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DERMAL, EYE AND ORAL TOXICOLOGIC EVALUATIONS, PHASE IV REPORT with Disperse Red 11, Disperse Blue 3, Solvent Red 1, and Red and Violet Mixtures

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Six test articles were evaluated to establish their eye and skin irritation potential and their oral and dermal toxicity. The test articles evaluated were as follows: (1) Disperse Red 11 - Lot 1; (2) Disperse Red 11 - Lot 2; (3) Disperse Blue 3; (4) Violet Mixture - 35 parts Disperse Red 11-Lot 1:5 parts Disperse Blue 3; (5) Solvent Red 1; and (6) Red Mixture - 33.4 parts Solvent Red 1:6.6 parts Disperse Red 11 - Lot 1. Oral studies were conducted utilizing the Fischer-344 albino rat as the test system; all other studies utilized the New Zealand White Albino Rabbit as the test system. The results obtained in these studies are summarized by test article below:

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Block 17 (cont.)

Field	Group
06	20

Block 18 (cont.)

Red Mix	CAS No. 2872-48-2; C.I.62015
Rabbit	CAS No. 2475-46-9; C.I.61505
Rat	CAS No. 1229-55-6; C.I.12150

Block 19 (cont.)

(1) Disperse Red 11 - Lot 1 was found to be a moderate skin irritant; tested negative for eye irritation; dermal LD50 >2g/kg; oral LD50 for males between 708 and 891mg/kg, for females >5g/kg.

(2) Disperse Red 11 - Lot 2 was found to be a mild skin irritant; tested negative for eye irritation; dermal LD50 >2g/kg; oral LD50 for males 1042.7 mg/kg, for females >5g/kg.

(3) Disperse Blue 3 was found to be practically non-irritating; tested negative for eye irritation; dermal LD50 >2g/kg; oral LD50 >5g/kg.

(4) Violet Mixture (35 parts Disperse Red 11 Lot 1:5 parts Disperse Blue) was found to be a mild skin irritant; tested negative for eye irritation; dermal LD50 >2g/kg; oral LD50 for males between 794 and 1000 mg/kg, for females between 1413 and 1778mg/kg, for combined sexes 1052mg/kg.

(5) Solvent Red 1 was found to be a non-irritating skin irritant; tested positive for eye irritation; dermal LD50 >2g/kg; oral LD50 >5g/kg.

(6) Red Mixture (33.4 parts Solvent Red 1:6.6 parts Disperse Red 11 Lot 1) was found to be a non-irritating skin irritant; tested positive for eye irritation; dermal LD50 >2g/kg; oral LD50 >5g/kg.

EXECUTIVE SUMMARY

Six test articles were evaluated to establish their eye and skin irritation potential and their oral and dermal toxicity. Four of the 6 test articles were provided for testing by the U.S. Army Medical Research and Development Command and the 2 remaining test articles were mixtures (prepared by this laboratory) of 2 of the materials provided. The test articles evaluated were as follows:

1) Disperse Red 11 - Lot 1
2) Disperse Red 11 - Lot 2
3) Disperse Blue 3
4) Violet Mixture - 35 parts Disperse Red 11 (Lot 1):5 parts Disperse Blue 3
5) Solvent Red 1
6) Red Mixture - 33.4 parts Solvent Red 1:6.6 parts Disperse Red 11 (Lot 1)

The tests conducted for each test article consisted of a standard, single dose Primary Eye Irritation Study, Primary Dermal Irritation Study, Acute Oral (5 g/kg) Toxicity Study, and an Acute Dermal (2 g/kg) Toxicity Study which included histologic examination of skin test sites for 2 animals per sex. Subsequently, Oral LD50 Studies were conducted with Disperse Red 11 - Lot 1; Disperse Red 11 - Lot 2; and Violet Mixture. Oral toxicity studies were conducted utilizing the Fischer-344 albino rat as the test system; all other studies utilized the New Zealand White albino rabbit as the test system. The results obtained in these studies are summarized, by test article, below followed by an overall summary provided in chart form.

- Disperse Red 11 - Lot 1 was found to be a moderate skin irritant (calculated primary irritation index was 2.7); tested negative for eye irritation; had a dermal LD50 greater than 2 g/kg. The oral LD50 for males was incalculable, however, based on the death patterns, the LD50 for males would be considered to be between 708 and 891 mg/kg. The oral LD50 for females is greater than 5 g/kg.

- Disperse Red 11 - Lot 2 was found to be a mild skin irritant (calculated primary irritation index was 0.73); tested negative for eye irritation; had a dermal LD50 greater than 2 g/kg; an oral LD50 of 1042.7 mg/kg in males, and greater than 5 g/kg for females.

- Disperse Blue 3 was found to be practically non-irritating to the skin (calculated primary irritation index was 0.5); tested negative for eye irritation; had a dermal LD50 greater than 2 g/kg; and an oral LD50 greater than 5 g/kg.

- Violet Mixture - 35 parts Disperse Red 11 (Lot 1) to 5 parts Disperse Blue 3 was found to be a mild skin irritant (calculated primary irritation index was 1.3); tested negative for eye irritation; had a dermal LD50 greater than 2 g/kg; and an oral LD50 of between 794 and 1,000 mg/kg for males, between 1,413 and 1,778 mg/kg for females, and 1,052 mg/kg for combined sexes.

- Solvent Red 1 was found to be non-irritating to the skin (calculated primary irritation index was 0.0); tested positive for eye irritation; had a dermal LD50 greater than 2 g/kg; and an oral LD50 greater than 5 g/kg.

- Red Mixture - 33.4 parts Solvent Red 1 to 6.6 parts Disperse Red 11 (Lot 1) was found to be non-irritating to the skin (calculated primary irritation index was 0.0); tested positive for eye irritation; had a dermal LD50 greater than 2 g/kg; and an oral LD50 greater than 5 g/kg.

SUMMARY OF RESULTS

TEST ARTICLE	PRIMARY DERMAL	PRIMARY EYE	ACUTE DERMAL	ACUTE ORAL
Disperse Red 11 - Lot 1	PII 2.7 (Moderately Irritating)	NEGATIVE	NT-RD (LD50>2 g/kg)	5 of 10 dead - all males (LD50-Males between 708 and 891 mg/kg* LD50-Females >5 g/kg)
Disperse Red 11 - Lot 2	PII 0.73 (Mildly Irritating)	NEGATIVE	NT-RD (LD50>2 g/kg)	5 of 10 dead - all males (LD50-Males 1042.7 mg/kg LD50-females >5 g/kg)
Disperse Blue 3	PII 0.5 (Practically Non-Irritating)	NEGATIVE	NT-RD (LD50>2 g/kg)	2 of 10 dead - 1 male, 1 female (LD50>5 g/kg)
Violet Mixture**	PII 1.3 (Mildly Irritating)	NEGATIVE	NT-RD (LD50>2 g/kg)	10 of 10 dead LD50-males - between 794 & 1000 mg/kg* LD50-females - between 1413 & 1778 mg/kg* LD50-combined sexes - 1052.0 mg/kg
Solvent Red 1	PII 0.0 (Non-Irritating)	POSITIVE	NT-RD (LD50>2 g/kg)	NT-RD (LD50>5 g/kg)
Red Mixture***	PII 0.0 (Non-Irritating)	POSITIVE	NT-RD (LD50>2 g/kg)	1 of 10 dead - male (LD50>5 g/kg)

KEY: PII denotes Primary Irritation Index
NT-RD denotes No Treatment-Related Deaths

* = LD50 not able to be calculated by computer.
** = 35 parts Disperse Red 11 (Lot 1):5 parts Disperse Blue 3.
*** = 33.4 parts Solvent Red 1:6.6 parts Disperse Red 11 (Lot 1).

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FOREWORD

All work relating to this study was done in conformity with the FDA Good Laboratory Practice Regulations.

Sandra H. Smith
Toxicologist — Date: 9/4/86

Dale A. Mayhew, Ph.D.
Principal Investigator — Date: 9/3/86

All work relating to this study was done in conformity with the FDA Good Laboratory Practice Regulations. The study was inspected during its progress, by a Quality Assurance Specialist according to ABC Standard Operating Procedure (SOP) for inspecting acute studies. Management was informed at once of any serious problems found.

The data in the report were compared with the raw data and are in agreement. The report and study file were examined to assure that any problems found during Quality Assurance inspections or audits were corrected, and if necessary, their effect on the study documented. (See Appendix A)

Antoinette Skelley
Manager, Quality Assurance
and Regulatory Affairs — Date: 9/4/86

All raw data relating to this study will be stored at ABC. Storage will conform to FDA regulations as per ABC SOP's and may include volume reduction by conversion to certified microform.

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INTRODUCTION

At the request of the U.S. Army Medical Research and Development Command, short term/standard acute studies were conducted on 6 test articles to evaluate their irritation (eye and skin) and toxicity (oral and dermal) potential. The materials tested were 1) Disperse Red 11 - Lot 1, 2) Disperse Red 11 - Lot 2, 3) Disperse Blue 3, 4) Solvent Red 1, 5) Violet Mixture, and 6) Red Mixture. Studies were conducted at American Biogenics Corporation (ABC), Decatur, IL facilities, from August 26, 1985 to December 11, 1985.

MATERIALS & METHODS

A. Test System/Husbandry

The study outline presents the study number, test article used, type of study, species, strain, supplier, body weight range, and duration for each study. Young adult animals were used for each study. The rat and rabbit are the species preferred for acute toxicological testing.

Animals were housed individually in stainless steel, wire-bottomed cages that conformed to the size standards specified in DHEW Publication (NIH) 78.23. The cages on each rack were numbered in a standard manner and a list of random numbers was generated by computer program* for each rack of cages. After receipt, each animal was removed from the shipping container and housed in the appropriate randomly selected cage. Each animal was then assigned a sequential animal number unique within American Biogenics Corporation (ABC) and identified with an ear tag bearing this animal number. The sequential animal number was listed on a cage card that was affixed to the front of the animal's cage.

The rabbits were quarantined for at least 14 days after receipt and the rats were quarantined for at least 7 days after receipt. Veterinary Sciences personnel observed the animals during quarantine for mortality, morbidity, and abnormal signs. Animals were examined during quarantine and only those considered to be in good health were used in these studies.

The quarantine and study rooms were cleaned daily and the cages were cleaned and sanitized as specified in ABC SOP's. Urine and feces fell through the wire mesh floor onto animal caging board. The cage boards were changed at least 2 to 3 times per week.

The animal room was well ventilated and air-conditioned. The temperature and humidity were monitored daily during the quarantine and study periods according to ABC SOP's. ABC temperature and humidity ranges for rabbits are 67 $\pm 5^{\circ}$F and 30-70%, respectively, and for rats are 73 $\pm 5^{\circ}$F and 30-70%, respectively. Any deviations in these ranges are noted in the raw data and were not considered to have effected the outcome of the studies.

The animal rooms were lighted from approximately 6:00 a.m. to 6:00 p.m. (12 hour light/dark cycle) using automatic timers.

Purina Certified Rodent Chow 5002, and Purina Certified Rabbit Chow 5322 were fed to the rats and rabbits, respectively, ad libitum during the quarantine and study periods except for fasting prior to dosing for rats only. Filtered tap water was provided ad libitum through an automatic watering system and was analyzed periodically as specified in ABC SOP's.

* Method adapted from Carnahan, Luther, and Wilkes, Applied Numerical Methods, Wiley, 1969.

STUDY OUTLINE

Study Number	Test Article	Type of Study	Species	Strain	Supplier	Body Weight Range at Study Start*	Duration of Study
488-2270	Disperse Red 11 - Lot 1	Primary Dermal Irritation	Albino rabbit	New Zealand White	K	2.00 - 2.30 kilograms	August 26, 1985 - September 3, 1985
488-2271	Disperse Red 11 - Lot 1	Primary Eye Irritation	Albino rabbit	New Zealand White	K	2.24 - 2.44 kilograms	August 27, 1985 - August 30, 1985
488-2272	Disperse Red 11 - Lot 1	Acute Oral Toxicity	Albino rat	Fischer F-344	CR	Males, 196-206 g Females, 138-143 g	September 11, 1985 - September 25, 1985
		Acute Oral LD50 Males only				Males, 175-236 g	October 1, 1985 - December 5, 1985
488-2273	Disperse Red 11 - Lot 1	Acute Dermal Toxicity	Albino Rabbit	New Zealand White	K	2.06 - 2.60 kilograms	September 5, 1985 - September 19, 1985
488-2274	Disperse Red 11 - Lot 2	Primary Dermal Irritation	Albino rabbit	New Zealand White	K	2.00 - 2.42 kilograms	August 26, 1985 - August 29, 1985
488-2275	Disperse Red 11 - Lot 2	Primary Eye Irritation	Albino rabbit	New Zealand White	K	2.20 - 2.30 kilograms	August 27, 1985 - August 30, 1985
488-2276	Disperse Red 11 - Lot 2	Acute Oral Toxicity	Albino rat	Fischer F-344	CR	Males, 186-202 g Females, 138-141 g	September 11, 1985 - September 25, 1985
		Acute Oral LD50 Males only				Males, 196-238 g	September 27, 1985 - November 26, 1985
488-2277	Disperse Red 11 - Lot 2	Acute Dermal Toxicity	Albino rabbit	New Zealand White	K	2.20 - 2.70 kilograms	September 5, 1985 - September 19, 1985
488-2278	Disperse Blue 3	Primary Dermal Irritation	Albino rabbit	New Zealand White	K	1.94 - 2.56 kilograms	August 26, 1985 - August 29, 1985
488-2279	Disperse Blue 3	Primary Eye Irritation	Albino rabbit	New Zealand White	K	2.06 - 2.52 kilograms	August 27, 1985 - August 30, 1985
488-2280	Disperse Blue 3	Acute Oral Toxicity	Albino rat	Fischer F-344	CR	Males, 186-205 g Females, 141-156 g	September 11, 1985 - September 25, 1985
488-2281	Disperse Blue 3	Acute Dermal Toxicity	Albino rabbit	New Zealand White	K	2.20 - 2.84 kilograms	September 4, 1985 - September 18, 1985

g = grams
K = Kuiper's Rabbit Ranch, Gary, IN
CR = Charles River Breeding Laboratories, Inc. (Kingston, NY facility)

STUDY OUTLINE (CONTINUED)

Study Number	Test Article	Type of Study	Species	Strain	Supplier	Body Weight Range at Study Start*	Duration of Study
480-2282	Violet Mixture	Primary Dermal Irritation	Albino rabbit	New Zealand White	K	2.20 - 3.00 kilograms	October 7, 1985 - October 11, 1985
480-2283	Violet Mixture	Primary Eye Irritation	Albino rabbit	New Zealand White	K	2.00 - 2.10 kilograms	October 7, 1985 - October 10, 1985
480-2284	Violet Mixture	Acute Oral Toxicity	Albino rat	Fischer F-344	CR	Males, 227-235 g Females, 162-172 g	October 9, 1985 - October 12, 1985
		Acute Oral LD50				Males, 175-237 g Females, 144-196 g	October 22, 1985 - December 11, 1985
480-2285	Violet Mixture	Acute Dermal Toxicity	Albino rabbit	New Zealand White	K	2.26 - 2.88 kilograms	October 15, 1985 - October 29, 1985
480-2286	Solvent Red 1	Primary Dermal Irritation	Albino rabbit	New Zealand White	K	2.50 - 3.10 kilograms	September 17, 1985 - September 20, 1985
480-2287	Solvent Red 1	Primary Eye Irritation	Albino rabbit	New Zealand White	K	2.40 - 2.50 kilograms	September 16, 1985 - September 24, 1985
480-2288	Solvent Red 1	Acute Oral Toxicity	Albino rat	Fischer F-344	CR	Males, 209-221 g Females, 174-183 g	October 30, 1985 - November 13, 1985
480-2289	Solvent Red 1	Acute Dermal Toxicity	Albino rabbit	New Zealand White	K	2.14 - 2.84 kilograms	September 12, 1985 - September 26, 1985
480-2290	Red Mixture	Primary Dermal Irritation	Albino rabbit	New Zealand White	K	2.50 - 2.74 kilograms	October 8, 1985 - October 11, 1985
480-2291	Red Mixture	Primary Eye Irritation	Albino rabbit	New Zealand White	K	2.28 - 2.64 kilograms	October 8, 1985 - October 29, 1985
480-2292	Red Mixture	Acute Oral Toxicity	Albino rat	Fischer F-344	CR	Males, 216-236 g Females, 166-178 g	October 10, 1985 - October 24, 1985
480-2293	Red Mixture	Acute Dermal Toxicity	Albino rabbit	New Zealand White	K	2.20 - 2.50 kilograms	November 12, 1985 - November 26, 1985

g = grams
K = Kuiper's Rabbit Ranch, Gary, IN
CR = Charles River Breeding Laboratories, Inc. (Portage, MY facility)

B. Test Articles

The identification and amount used of each test article are listed below:

ABC Code Number	Sponsor Identification	Amount of Test Article Used
7/85-1047	Disperse Red 11 - Lot 1 (CAS No. 2872-48-2)(C.I.62015)	258.961 grams
7/85-1048	Disperse Red 11 - Lot 2 (CAS No. 2872-48-2)(C.I.62015)	95.290 grams
7/85-1049	Disperse Blue 3 (CAS No. 2475-46-9)(C.I.61505)	96.060 grams
7/85-1050	Solvent Red 1 (CAS No. 1229-55-6)(C.I.12150)	196.880 grams
7/85-1047+ 7/85-1048	Violet Mixture	N/A
7/85-1047+ 7/85-1050	Red Mixture	N/A

N/A = Not applicable; included in the individual material quantities given above.

+ Mixtures were prepared using a weight/weight ratio.

Test articles (ABC Code Nos. 7/85-1047,1048,1049,1050) were provided by the Sponsor (U.S. Army Medical Research and Development Command). Records concerning the test article purity, source, and other data required by GLP's are the responsibility of the Sponsor. The test articles were stored under ambient conditions at this laboratory.

The usages of the different test articles are described as follows:

Disperse Red 11 - Lot 1 -

- Moistened with physiological saline prior to application, 0.5 gram per patch, and test sites wiped off with water following application/exposure interval for Primary Dermal Irritation Test.

- Moistened with physiological saline prior to application and test sites wiped off using physiological saline following application/exposure interval for Acute Dermal Toxicity Test. The amount of test article applied per square centimeter for each animal was calculated to be 21.5 milligrams for the Acute Dermal Toxicity Test.

- Suspended in corn oil using a 30% weight/volume ratio for administration of the Acute Oral Toxicity Test.

- Suspended in corn oil using a constant volume of 17 ml/kg for administration of the acute oral LD50 Test.

- Instilled neat, 0.1 gram per eye, for the Primary Eye Irritation Test.

Disperse Red 11 - Lot 2 -

- Moistened with physiological saline prior to application, 0.5 gram per patch, and test site wiped off using physiological saline following application/exposure interval for the Primary Dermal Irritation Test.

- Moistened with physiological saline prior to application and test sites wiped off using physiological saline following the application/exposure interval for the Acute Dermal Toxicity Test. The amount of test article applied per square centimeter for each animal was calculated to be 21.7 milligrams for the Acute Dermal Toxicity Test.

- Suspended in deionized water using a 30% weight/volume ratio for administration of the Acute Oral Toxicity Test.

- Suspended in corn oil using a constant volume of 17 ml/kg for administration of the Acute Oral LD50 Test.

- Instilled neat, 0.1 gram per eye, for Primary Eye Irritation Test.

Disperse Blue 3 -

- Moistened with physiological saline prior to application, 0.5 gram per patch, and test sites wiped off following application/exposure interval using physiological saline for the Primary Dermal Irritation Test.

- Moistened with physiological saline prior to application and sites wiped off following application/exposure interval using physiological saline for the Acute Dermal Toxicity Test. The amount of test article applied per square centimeter for each animal was calculated to be 22.2 milligrams for the Acute Dermal Toxicity Test.

- Suspended in corn oil using a 30% weight/volume ratio for administration of the Acute Oral Toxicity Test.

- Instilled neat, 0.1 gram per eye, for the Primary Eye Irritation Test.

Violet Mixture - 35 parts Disperse Red 11 - Lot 1:5 parts Disperse Blue 3 - The mixture was prepared prior to each use.

- Moistened with physiological saline prior to application, 0.5 gram per patch, and test sites wiped off following application/exposure interval using physiological saline for the Primary Dermal Irritation Test.

- Moistened with physiological saline prior to application and sites wiped off following application/ exposure interval using physiological saline for the Acute Dermal Toxicity Test. The amount of test article applied per square centimeter for each animal was calculated to be 22.1 milligrams for the Acute Dermal Toxicity Test.

- Suspended in corn oil using a 30% weight/volume ratio for administration of the Acute Oral Toxicity Test.

- Suspended in corn oil using a constant volume of 17 ml/kg for administration of the Acute Oral LD50 test.

- Instilled neat, 0.1 gram per eye for the Primary Eye Irritation Test.

Solvent Red 1 -

- Moistened with physiological saline prior to application, 0.5 gram per patch, and test sites wiped off following application/exposure interval using physiological saline for the Primary Dermal Irritation Test.

- Moistened with physiological saline prior to application and sites wiped off following application/exposure interval using physiological saline for the Acute Dermal Toxicity Test. The amount of test article applied per square centimeter for each animal was calculated to be 21.0 milligrams for the Acute Dermal Toxicity Test.

- Suspended in corn oil using a 18% weight/volume ratio for administration of the Acute Oral Toxicity Test.

- Instilled neat, 0.1 gram per eye for the Primary Eye Irritation Test.

Red Mixture - 33.4 parts Solvent Red 1:6.6 parts Disperse Red 11 - Lot 1 - The mixture was prepared prior to each use.

- Moistened with physiological saline prior to application, 0.5 gram per patch, and test sites wiped off following application/exposure interval using physiological saline for the Primary Dermal Irritation Test.

- Moistened with physiological saline prior to application and sites wiped off following application/exposure interval using physiological saline for the Acute Dermal Toxicity Test. The amount of test article applied per square centimeter for each animal was calculated to be 20.0 milligrams for the Acute Dermal Toxicity Test.

- Suspended in corn oil using a 20% weight/volume ratio for administration of the Acute Oral Toxicity Test.

- Instilled neat, 0.1 gram per eye for the Primary Eye Irritation Test.

C. Experimental Design

Each test and the procedures thereof for each test article are described below.

1. Primary Dermal Irritation

The duration of the study was at least 72 hours after test article application, but not more than 21 days. See Study Outline (page 9) for the specific duration of each study. Groups consisting of 3 male and 3 female rabbits were used.

Animals were assigned to each study by sequential animal number except for the elimination of any animal deemed unsuitable. Two (2) application sites were prepared on either side of the thoracic region spinal column of each animal by closely clipping the hair with Oster electric clippers equipped with a number 40 (surgical) blade; the prepared sites were examined and only animals free of dermal lesions/irritations were assigned to each study.

The right anterior and left posterior application sites were abraded with a needle to penetrate the stratum corneum but not the dermis. The other application sites were left intact. One-half (0.5) gram of a solid was moistened with physiological saline and applied to each 2.5 centimeter square gauze patch. While each animal was manually immobilized, 1 patch containing the test article was applied to each application site and held in place with gauze wrapping. The entire trunk of each animal was then wrapped with plastic wrap and stockinette.

After 24 hours of exposure, the bandage and patches were removed. Each test site was gently wiped with gauze sponges moistened with an appropriate vehicle (known not to cause any dermal toxic reactions) to remove any remaining test article. The skin condition of each test site was evaluated for erythema, edema, and other lesions at 24 and 72 hours ($\pm$2 hours at each interval) and daily thereafter to day 21 or until irritation had subsided. Dermal reaction scores were assigned using the grading system presented in Appendix B of this report.

Each animal was weighed prior to application of each test article.

All animals were observed at least once daily for mortality and obvious toxic signs.

At the termination of each study, animals were euthanized by administration of an intravenous injection of T-61 Euthanasia Solution and discarded.

The Primary Irritation Score was calculated for each test article as follows: the average scores for erythema and eschar formation for intact and abraded skin at 24 ($\pm$2) and 72 ($\pm$2) hours were added to the average scores for edema for intact and abraded skin at 24 ($\pm$2) and 72 ($\pm$2) hours. The total of the 16 values was divided by 8 to give the Primary Irritation Score.

Based upon the mean Primary Irritation Score, the test article was given a descriptive irritation rating using the following method:

Mean Primary Irritation Score (Range of Values)	Descriptive Reading
0.0	Non-irritating
0.1 - 0.5	Practically non-irritating
0.6 - 2.0	Mildly irritating
2.1 - 5.4	Moderately irritating
5.5 and above	Severely irritating

2. Primary Eye Irritation

The duration of the study was at least 72 hours after instillation, but not more than 21 days. See the Study Outline for the specific duration for each study. Groups consisting of 3 rabbits, male or female, were used.

Both eyes of each animal were evaluated within 24 hours prior to instillation of each test article using the evaluation system presented in Appendix C. Animals were assigned to the study by sequential animal number. However, any animal deemed unsuitable was not used and the next acceptable sequentially numbered animal was used.

One hundred (100) milligrams of a solid test article was instilled onto the everted lower lid of the right eye of each animal. The upper and lower lids were held together for approximately 1 second to prevent loss of the test article and to ensure even distribution of the test article over the surface of the eye.

The treated eye of each animal was examined for ocular irritation and lesions at 24, 48 and 72 hours (±2 hours at each interval). The treated eyes were evaluated on days 7, 14, and 21 if irritation persisted. Each study was terminated after 21 days or when irritation subsided after the 72 hour evaluations. The evaluation system presented in Appendix C of the report was used for scoring ocular reactions. A pocket flashlight without magnification and a 2 percent sodium fluorescein solution in deionized water were used at all evaluation intervals.

Each animal was weighed prior to instillation of each test article.

The animals were observed at least once daily for mortality and obvious toxic signs.

All animals were sacrificed at the termination of the study by administration of an intravenous injection of T-61 Euthanasia Solution and discarded.

The mean Primary Eye Irritation Score with standard deviation and standard error values were calculated at each evaluation interval using the total scores of each treated eye.

An animal was considered to have exhibited a positive response if the test article produced one or more of the following signs:

- ulceration of the cornea
- opacity of the cornea
- inflammation of the iris
- obvious swelling in the conjunctivae with partial eversion of the eyelid
- a diffuse crimson color

3. Acute Oral/Acute Oral LD50 Toxicity

The duration of each study was 14 days after dosing. Groups consisting of 5 male and 5 female rats were used for each test article/test group. If deaths occurred in the Acute Oral Study, a 7-day range-finding test was conducted using 5 groups consisting of 1 male and/or 1 female in each group to set levels for the Acute Oral LD50 Study.

Animals were assigned to each study by sequential animal number. However, any animal deemed unsuitable was not used and the next acceptable sequentially numbered animal was used.

The animals were fasted overnight. The following morning, body weights were recorded, doses were calculated, and a measured volume of the test article suspension was delivered to each animal by oral gavage in a single dose for aqueous suspensions, and in 2 doses for non-aqueous suspensions. Diet was returned to the cage of each animal immediately after administration.

Animals were observed frequently for mortality and toxic signs after dosing on day 0. Thereafter, observations for mortality and toxic signs were done at least once daily. Body weights were recorded prior to test article administration on day 0, and on days 3, 7, 10 and prior to sacrifice on day 14, or at the time found dead.

On day 14, all surviving animals were rendered unconscious by exposure to carbon dioxide and exsanguinated prior to necropsy; those that succumbed were necropsied as soon as possible after death was noted. The following of each animal were examined and all abnormal findings were recorded: all external surfaces and orifices, and abdominal, thoracic, and pelvic cavities and their viscera. Necropsies were conducted under the supervision of a pathologist. Range-finding animals were discarded at termination. The mean, standard deviation, and standard error were calculated for the body weight data and for the amount of test article administered.

The oral LD50 value, the 95 percent confidence interval, the slope of the dose-response curve, and correction factors for 0 and 100 percent observed responses were calculated by computer program employing the methodology of Litchfield and Wilcoxon.+

+ Litchfield, J. T., Jr., and Wilcoxon, F., "A Simplified Method of Evaluating Dose-Effect Experiments", Journal of Pharmacology and Experimental Therapeutics, Vol. 96, 1949, pages 99-113.

4. Acute Dermal Toxicity

The duration of each study was 14 days after test article application. Groups consisting of 5 male and 5 female rabbits were used for each test article.

Animals were assigned to the study by sequential animal number. However, any animal deemed unsuitable, including any animal exhibiting dermal lesions, was not used and the next acceptable sequentially numbered animal was used.

The dorsal and lateral trunk (approximately 10% of the body surface area) of each animal was clipped free of hair with Oster electric clippers equipped with a number 40 (surgical) blade.

On the day of dosing, body weights were recorded and doses were calculated. Approximately 24 (+2) hours after clipping, each animal received longitudinal abrasions every 2 to 3 cm. The abrasions were deep enough to penetrate the stratum corneum, but not the dermis. The test articles at 2 g/kg were applied on a 6 inch by 6 inch pad and placed over the dorsal surface area. Solid test articles were moistened with physiological saline to form a paste and applied to the pad. The test article was held in contact with the skin with gauze wrapping. The entire trunk was then wrapped with plastic wrap and stockinette.

After 24 hours of exposure, the bandage and pads were removed. Each application site was gently wiped with gauze sponges moistened with an appropriate vehicle (known not to cause any dermal toxic reactions) to remove any remaining test article.

All animals were observed for mortality and abnormal clinical signs frequently after dosing on day 0. Thereafter, observations for mortality and abnormal signs were done at least once daily. Body weights were recorded prior to test article application on day 0, and on days 3, 7, 10, and prior to sacrifice on day 14, or at the time found dead.

On day 14, all surviving animals were rendered unconscious by administration of injections of a barbiturate and exsanguinated prior to necropsy; those that succumbed were necropsied as soon as possible after death was noted. The following of each animal were examined and all abnormal findings were recorded: all external surfaces and orifices, and abdominal, thoracic, and pelvic cavities and their viscera. Necropsies were conducted under the supervision of a pathologist. The treated skin and corresponding untreated control skin was saved for histopathological examination. No other tissues were saved.

Histopathological examination was performed on animals that had succumbed during the test period, and on 2 animals per sex necropsied at the end of the test period. Examinations included skin from the treated and corresponding untreated sites.

The mean, standard deviation, and standard error were calculated for the body weight data and for the amount of test article applied. The approximate amount of test article applied was calculated in milligrams per square centimeter of exposed skin.

RESULTS

A. Primary Dermal Irritation Studies

Disperse Red 11 - Lot 1

Individual body weights and dermal reaction scores recorded during the study are given in Table 1.

No mortalities or abnormal clinical signs were observed during the study.

Erythema (grades 1 through 3), edema (grades 1 through 3), and desquamation were observed during the study. All dermal irritation had subsided by the day 7 evaluation. The calculated Primary Irritation Score was 2.7; the test article was considered to be moderately irritating.

Disperse Red 11 - Lot 2

Individual body weights and dermal reaction scores recorded during the study are given in Table 2.

No moralities and no abnormal clinicial signs were observed during the study. Erythema and edema (grades 2 and 1) was observed at the 24 and 72 hour evaluation intervals. Desquamation was observed at one test site at the 72 hour and day 4 evaluation interval. All dermal irritation had subsided by the day 4 evaluation.

The calculated Primary Irritation Score was 0.73; the test article was considered to be mildly irritating.

Disperse Blue 3

Individual body weights and dermal reaction scores recorded during the study are given in Table 3.

No mortalities or abnormal clinical signs were observed during the study.

Erythema (grades 1 and 2) and edema (grade 1), were noted during the study. All dermal irritation had subsided by the 72 hour evaluation. The calculated Primary Irritation Score was 0.5; the test article was considered to be practically non-irritating.

Violet Mixture

Individual body weights and dermal reaction scores recorded during the study are given in Table 4.

No mortalities occurred during the study. All animals exhibited purple-colored urine on day 2 after application. No other abnormal clinical signs were observed.

Erythema (grades 1 through 3), and edema (grades 1 or 2) were observed during the study. All dermal irritation had subsided by the day 4 evaluation. The calculated Primary Irritation score was 1.3; the test article was considered to be mildly irritating.

Solvent Red 1

Individual body weights and dermal reaction scores recorded during the study are given in Table 5.

No mortalities or abnormal clinical signs were observed during the study.

No dermal irritation was observed during the study. The calculated Primary Irritation Score was 0.0; the test article was considered to be non-irritating.

Red Mixture

Individual body weights and dermal reaction scores recorded during the study are given in Table 6.

No mortalities or abnormal clinical signs were observed during the study.

No dermal irritation was observed during the study. The calculated Primary Irritation Score was 0.0; the test article was considered to be non-irritating.

B. Primary Eye Irritation Studies

Disperse Red 11 - Lot 1

Individual body weights and ocular reaction scores recorded during the study are given in Table 7.

No mortalities or abnormal clinical signs were observed during the study.

No ocular irritation was observed during the study.

All animals were considered to have exhibited a negative response to the test article.

Disperse Red 11 - Lot 2

Individual body weights and ocular reaction scores recorded during the study are given in Table 8.

No mortalities or abnormal clinical signs were observed during the study.

Redness (grade 1) and chemosis (grade 1) was observed at the 24 hour evaluation. All ocular irritation had subsided by the 48 hour evaluation.

All animals were considered to have exhibited a negative response to the test article.

Disperse Blue 3

Individual body weights and ocular reaction scores recorded during the study are given in Table 9.

No mortalities or abnormal clinical signs were observed during the study.

No ocular irritation was observed during the study. All animals were considered to have exhibited a negative response to the test article.

Violet Mixture

Individual body weights and ocular reaction scores recorded during the study are given in Table 10.

No mortalities occurred during the study. All animals exhibited purple colored urine on days 1 and 2 after instillation. No other abnormal clinical signs were noted.

Two animals exhibited redness (grade 1) and chemosis (grade 1) at 24 hours after instillation. Ocular reactions had subsided by the 48 hour evaluation.

All animals were considered to have exhibited a negative response to the test article.

Solvent Red 1

Individual body weights and ocular reaction scores recorded during the study are given in Table 11.

No mortalities or abnormal clinical signs were observed during the study.

Redness (grades 1 through 3), chemosis (grades 1 and 2), discharge (grades 1 and 2), and iritis (grade 1) were observed during the study. Blistering of the conjunctivae was noted for all animals. Two of the animals also exhibited opacities and positive fluorescein staining. All ocular irritation had subsided by the day 7 evaluation.

All animals were considered to have exhibited a positive response to the test article.

Red Mixture

Individual body weights and ocular reaction scores recorded during the study are given in Table 12.

No mortalities or abnormal clinical signs were observed during the study.

Redness (grades 1 through 3), chemosis (grades 1 through 3), discharge (grades 1 through 3), iritis (grade 1), blistering of the conjunctivae, opacities, and positive fluorescein staining were observed during the study. Nicks in the upper and lower lids were noted for 1 animal. All ocular irritation had subsided for 2 of the animals by the day 7 evaluation. An opacity was still noted for 1 animal at the day 21 evaluation.

All animals were considered to have exhibited a positive response to the test article.

C. Acute Dermal Toxicity Studies

Disperse Red 11 - Lot 1

Individual data (body weight, test article administration, antemortem observations, and necropsy findings) are presented in Tables 13, 14, and 15. Histopathology results are given in Appendix D.

All animals survived the 14 day observation period of the study. All but one animal (male) gained weight. Observations noted during the study included: erythema; edema; test site, feet, and muzzle discolored purple; purple colored urine; loose stools; and/or yellow/brown stained fur in the perianal region.

Necropsy examinations of all animals revealed purple discolorations of the treated skin, fur of feet, fur of abdomen, and/or face; and red linear discoloration of anterior portion of treated skin.

The dermal LD50 of the test article was considered to be greater than 2 grams per kilogram of body weight.

Disperse Red 11 - Lot 2

Individual data (body weight, test article administration, antemortem observations, and necropsy findings) are presented in Tables 16, 17, and 18. Histopathology results are given in Appendix D.

Nine of ten animals survived the 14 day observation period of the study. One surviving male lost weight; all other surviving animals exhibited body weight gains. One male animal was found dead on day 14. Observations noted during the study included: violet colored urine; test site, feet, and muzzle stained violet; and death.

Necropsy examination of the found dead animal revealed: lungs with diffuse, red, firm consolidation and mild, tan exudate in the right lobes, treated skin with scattered purple areas, and pink discoloration of the fur on the feet.

Necropsy examination of the surviving animals at the final sacrifice revealed pink or purple discoloration of the feet and treated skin with scattered purple areas. One animal had a solitary raised purple discoloration on the treated skin and another animal had the urinary bladder distended with fluid.

The dermal LD50 of the test article was considered to be greater than 2 grams per kilogram of body weight.

Disperse Blue 3

Individual data (body weight, test article administration, antemortem observations, and necropsy findings) are presented in Tables 19, 20, and 21. Histopathology results are given in Appendix D.

All animals survived the 14 day observation period of the study and gained overall body weight. Observations noted during the study included 1 animal with few stools and all animals with test site, head, and feet stained blue.

Necropsy examinations of all animals revealed discoloration of the treated skin and of the fur on the feet and face.

The dermal LD50 of the test article was considered to be greater than 2 grams per kilogram of body weight.

Violet Mixture

Individual data (body weight, test article administration, antemortem observations, and necropsy findings) are presented in Tables 22, 23, and 24. Histopathology results are given in Appendix D.

All animals survived the 14 day observation period of the study. Eight of ten animals gained body weight over the same period. Two females exhibited body weight losses. Observations noted during the study included: test site, nose, and feet discolored purple, purple colored urine, loose stools, few stools, no urine, no stools, mucous-like stools, food appeared undisturbed, pale, animal appeared to be bloated at abdominal region, and emaciated.

Necropsy examinations of all animals revealed: purple discoloration of treated skin, fur of feet and/or fur of perineum; multiple depressions of kidneys; small intestine distended with fluid contents; or purple-tinged fluid in urinary bladder.

The dermal LD50 of the test article was considered to be greater than 2 grams per kilogram of body weight.

Solvent Red 1

Individual data (body weight, test article administration, antemortem observations, and necropsy findings) are presented in Tables 25, 26, and 27. Histopathology results are given in Appendix D.

All animals survived the 14 day observation period of the study and gained overall body weight. Observations noted during the study included: test site, feet, and head discolored red; few stools; and loose stool.

Necropsy examinations of all animals revealed red discoloration of treated skin, red discoloration of fur, a liver with a dark red discoloration, and 2 lungs with red discoloration.

The dermal LD50 of the test article was considered to be greater than 2 grams per kilogram of body weight.

Red Mixture

Individual data (body weight, test article administration, antemortem observations, and necropsy findings) are presented in Tables 28, 29, and 30. Histopathology results are given in Appendix D.

All animals survived the 14 day observation period of the study. One female lost weight; all other animals exhibited body weight gains. Observations noted during the study included: test sites, heads, feet, and abdomens stained red; few stools; loose stool; and yellow/brown stained fur in the perianal region.

Necropsy examinations of all animals revealed pink discolorations of the fur or skin on or near the treated sites.

The dermal LD50 of the test article was considered to be greater than 2 grams per kilogram of body weight.

D. Acute Oral Toxicity Studies

Disperse Red 11 - Lot 1

The individual data (body weight, test article administration, antemortem observations, and necropsy findings) are presented in Tables 31, 32, and 33.

All female animals survived the 14 day observation period of the study. All males died as early as day 4 and as late as day 6 of the study. Observations noted during the study included: purple colored urine, purple colored fur in the perianal region, loose stools, skin discolored purple, crusty eyes, crusty nose, lethargy, poor coat quality, purple colored tail, and death.

Necropsy examinations of the animals found dead revealed: pink or blue-pink discolored posterior extremities; pink/pale pink colored fat; green, dark grey, mottled, and/or granular appearance of liver; stomach filled with dark fluid; intestines contained dark fluid; pink colored testicular fat; bladder distended, filled with pink fluid; musculature pink or blue-pink in color; black discolorations of glandular stomach; pale

pancreas; pink discoloration of testes; hemorrhage of subcutaneous tissues of bilateral posterior appendage, left anterior appendage, and head; and pink/pale pink discoloration of skin.

Necropsy examinations of the surviving animals revealed purple stained fur in the perineum.

The acute oral LD50 of the test material for females was considered to be greater than 5 grams per kilogram of body weight. The acute oral LD50 of the test material for males is reported separately (see Section V.E. [Oral LD50 Studies] of this report).

Disperse Red 11 - Lot 2

The individual data (body weight, test article administration, antemortem observations, and necropsy findings) are presented in Tables 34, 35, and 36.

Five females survived the 14 day observation period of the study. All males died on days 4 and 5 of the study. Observations noted during the study included: red colored urine, skin discolored pink, dark colored stool, lethargy, ataxia, prostration, irregular breathing, lacrimation, crusty eye, crusty muzzle, few stools, gasping, red stained fur and tail, and death.

Necropsy examinations of the animals found dead revealed: livers with a granular appearance and/or discolored green or grey-green; red or brown discolorations of the glandular stomach mucosa; gastrointestinal tracts filled with thick and/or dark substance; lungs filled with dark substance; urinary bladders distended and/or filled with purple or pink fluid; pink colored fat; severe subcutaneous hemorrhage of appendages; abdominal cavity filled with red fluid; pink/pale pink musculature and skin; a swollen, dark blue foot; red fluid around nose and mouth; red, crusty material around nose, mouth, and ear; blue or blue-pink extremities; and perianal region covered with purple fluid.

Necropsy examinations of the surviving animals revealed a diaphragmatic hernia and pink stained fur in the perianal region.

The acute oral LD50 of the test material for females was considered to be greater than 5 grams per kilogram of body weight. The acute oral LD50 of the test material for males is reported separately (see Section V.E. [Oral LD50 Studies] of this report).

Disperse Blue 3

The individual data (body weight, test article administration, antemortem observations, and necropsy findings) are presented in Tables 37, 38, and 39.

Eight of ten animals survived the 14 day observation period of the study. One male animal was found dead on day 7 and a female animal was found dead on day 3. Observations noted during the study included: blue colored urine, skin discolored blue, crusty nose, emaciation, poor coat quality, crusty eyes, sensitive to touch, blue stained tail, lethargy, ataxia, blue stained fur in the perianal region, and death.

Necropsy examinations of the animals found dead revealed: livers pale or discolored grey green, stomach stained blue, gastrointestinal contents dark, musculature with varying degrees

of blue discoloration, urinary bladder containing purple fluid, cervical lymph node discolored red, posterior appendages with subcutaneous hemorrhages, skin discolored blue, and testicular fat discolored grey.

Necropsy examination at final sacrifice revealed livers discolored dark green or brown, testicular fat in males discolored grey, fat in females discolored grey, scattered purple stained areas on tail, and purple stained fur on the perineum.

The acute oral LD50 of the test material was considered to be greater than 5 grams per kilogram of body weight for male and female rats.

Violet Mixture

The individual data (body weight, test article administration, antemortem observations, and necropsy findings) are presented in Tables 40, 41, and 42.

All animals were found dead as early as 2 days and as late as 3 days after 5 g/kg dose administration. Observations noted during the study included: purple colored skin, purple colored urine, crusty eye, crusty nose, purple colored fur in the perianal region, lethargy, ataxia, and death.

Necropsy examinations of all animals revealed: intensely red or purple scattered areas, pale lungs; dark brown, mottled, grey, red, dark, and/or prominent lobular pattern of livers; appendages purple in color; skin dark purple; body fat dark purple; mucocutaneous junctions, gastrointestinal tract, upper respiratory tract, feet, gastrointestinal contents, mesenteric surface and cornea discolored purple.

The acute oral LD50 of the test material was considered to be less than 5 grams per kilogram of body weight, and is reported separately (see Section V.E. [Oral LD50 Studies] of this report).

Solvent Red 1

The individual data (body weight, test article administration, antemortem observations, and necropsy findings) are presented in Tables 43, 44, and 45.

All animals survived the 14 day observation period of the study and gained overall body weight. Observations noted during the study included: red colored urine and stool; loose stool; red stained fur in the perianal region, on the head and ventral portion of the body, and on the muzzle; and red colored stain on the tail.

Necropsy examinations of all animals revealed no abnormal findings.

The oral LD50 of the test material was considered to be greater than 5 grams per kilogram of body weight for male and female rats.

Red Mixture

The individual data (body weight, test article administration, antemortem observations, and necropsy findings) are presented in Tables 46, 47, and 48.

All but 1 animal survived the 14 day observation period of the study. One male was found dead on day 4 of the study. Observations noted during the study included: red-violet colored urine; red colored stool; loose stool; skin discolored pink; red stain on tail, feet, and muzzle; red stained fur in the perianal region, on the perineum, and on the ventral and dorsal body surfaces; lethargy; scabby tail; crusty nose and eye; poor coat quality; and death.

Necropsy examination of the animal found dead revealed a pale lung, contents of the gastrointestinal tract discolored red, fat and mesentery discolored red, crusted external surface of feet and tail, and contents of the urinary bladder discolored red.

Necropsy examinations of the surviving animals revealed purple staining of the perineum and red discoloration of lungs.

The oral LD50 of the test material was considered to be greater than 5 grams per kilogram of body weight for male and female rats.

E. Oral LD50 Studies

Disperse Red 11 - Lot 1

The individual data (body weight and test article administration, antemortem observations, and necropsy findings) are presented in Tables 49, 50, and 51. Table 52 presents the results of the Litchfield-Wilcoxon LD50 determinations.

Preliminary range-finding trials were conducted using 1 male at each of the following dose levels: 1,000, 1,413, 1,995, 2,818, and 3,981 milligrams per kilogram of body weight. Deaths occurred in all but the 1,413 mg/kg level; therefore, dose levels of 562, 708, 891, and 1,413 were selected for the LD50 determination.

The oral LD50 of Disperse Red 11 - Lot 1 for males was incalculable. Based on the death patterns, the LD50 for males would be considered to be between 708 and 891 mg/kg of body weight. The incidences of death were as follows:

Group (mg/kg)	Number Dead/Number Tested Males
562	0/5
708	0/5
891	4/5
1,413	5/5

All deaths occurred within 4 to 6 days after dosing.

Observations noted during the study included: purple colored urine, skin discolored purple, purple stained fur - perianal region, purple loose stools, loose stools, crusty eye, lethargy, red crusty substance around ear tag, crusty substance around ear tag, crusty nose, ataxia, squinting, pale, irregular breathing, no stools, red stained fur - all feet; slow respirations, prostration, sensitive to touch, and death.

Necropsy examinations of the animals found dead revealed: discolorations and/or prominent lobular pattern of livers; fat; discolorations of perineum, perianal region and base of tail; hemorrhage of subcutaneous tissues of appendages, musculature of appendages, tissue along descending aorta and along abdominal aorta; discolorations of epididymides; discoloration of testes, discoloration, hemorrhage, or pale lungs; dark or purple contents of gastrointestinal tract; pink, red or purple contents, distended, and/or discoloration of urinary bladder; clotted blood around ear tag; pink-red discoloration of anus; stomach contained copious red material; red brown crusted material around nose, mouth, on fat, and in perianal region; discoloration of non-glandular stomach; discoloration of spleen; discoloration of glandular stomach; and discoloration of small intestine.

Necropsy examinations of the surviving animals revealed purple stained fur of the perianal region or perineum; and discolorations of liver.

Disperse Red 11 - Lot 2

The individual data (body weight and test article administration, antemortem observations, and necropsy findings) are presented in Tables 53, 54, and 55. Table 56 presents the results of the Litchfield-Wilcoxon LD50 determinations. Figure E-1, on page 129, depicts the dose-response curve.

Preliminary range-finding trials were conducted using 1 male at each of the following dose levels: 1,000, 1,413, 1,995, 2,818, and 3,981 milligrams per kilogram of body weight. Deaths occurred in the 2,818 and 3,981 mg/kg dose levels only; therefore, dose levels of 562, 891, and 1,413 mg/kg were selected for the LD50 determinations.

The oral LD50 of Disperse Red 11 - Lot 2 was determined to be 1,042.7 mg/kg for males. The incidences of death were as follows:

Group (mg/kg)	Number Dead/Number Tested Males
562	0/5
891	3/5
1,413	3/5

All deaths occurred within 5 to 6 days after dosing.

Observations noted during the study included: red-violet colored urine; red-violet colored stool; skin discolored pink; loose stool; no stool; few stools; lethargy; ataxia; pale; squinting; poor coat quality; red-violet stain on fur, tail, or muzzle; red stain on tail; crusty eye; crusty nose; crusty muzzle; crusty substance around ear tag; red discharge from penis or on foot; scab on foot; yellow/brown stained fur in the perianal region; and death.

Necropsy examinations of the animals found dead revealed: green, grey, or dark discoloration of the liver; dark or purple contents in the gastrointestinal tract; pink colored fat, fur,

appendages, and tail; pelvic and abdominal cavities and viscera with purple-pink discoloration; pale kidney cortices, lung, and skeletal muscle; subcapsular hemorrhage of testes; red discoloration of testes; urinary bladder distended with pink fluid; urinary bladder containing dark purple fluid; appendages/extremities with subcutaneous red discoloration or hemorrhage; purple-pink or crusted black discoloration on pelvic region; and red or red brown crusty material around nose and mouth.

Necropsy examinations of the surviving animals revealed: grey, dark green, or green-grey discoloration of the liver; pink or purple-pink discoloration of fur in the pelvic region; purple stained fur in the perianal region; and a crusted scab anterior to the penis.

Violet Mixture

The individual data (body weight, test article administration, antemortem observations, and necropsy findings) are presented in Tables 57, 58, and 59. Tables 60, 61, and 62 present the results of the Litchfield-Wilcoxon LD50 determinations. Figure E-2, on page 141, depicts the dose response curve of combined sexes.

Preliminary range-finding trials were conducted using 1 male and 1 female in each of the following dose levels: 447, 794, 1,413, 2,512, and 4,467 milligrams per kilogram of body weight. Deaths occurred to both male and female animals of the 2,512 and 4,467 mg/kg levels. Deaths also occurred in the males only receiving 794 and 1,413 mg/kg. Therefore, dose groups 1,000, 1,413, and 1,995 mg/kg were initially selected, then two additional groups were used; 794 mg/kg for males only, and 1,778 mg/kg for females only, for LD50 determinations.

The oral LD50 of Violet Mixture for males was unable to be calculated. Based on the death patterns, the LD50 would be considered to be between 794 and 1,000 mg/kg of body weight. The oral LD50 for females was unable to be calculated. Based on the death patterns, the LD50 would be considered to be beweeen 1,413 and 1,778 mg/kg of body weight. The oral LD50 of combined sexes was calculated to be 1,052.0 mg/kg of body weight. The incidences of death were as follows:

Group (mg/kg)	Number Dead/Number Tested Males	Females	Combined Sexes
794	1/5	-	1/5
1,000	5/5	0/5	5/10
1,413	5/5	2/5	7/10
1,778	-	5/5	5/5
1,995	5/5	5/5	10/10

All deaths occurred within 2 to 7 days after dosing.

Observations noted during the study included: purple colored urine, purple colored loose stools, purple stained fur - perianal region, skin discolored purple, crusty nose, crusty eye,

lethargy, few stools, sensitive to touch, loose stools, no stools, ataxia, alopecia - posterior legs, squinting, prostration, labored respirations, lacrimation, irregular breathing, body cool to touch, and death.

Necropsy examinations of the animals found dead revealed: discolorations, pale, and/or prominent or exaggerated lobular pattern of livers; dark, purple, and/or black contents of gastrointestinal tract; discolorations of body fat; discolorations of glandular stomach; hemorrhages of subcutaneous or musculature of appendages or body; purple discolorations of fur and/or skin of pelvic region, tail and/or appendages; dried blood or black crusty material around ear tag, muzzle, nose and/or mouth; abdominal and/or thoracic cavities contained red or blood contents; urinary bladder contained purple fluid; prostate discolored purple; urine discolored purple; intestine discolored purple; red discolorations and/or mottling of lungs; dark red discoloration of thymus; and purple testes.

Necropsy examinations of the surviving animals revealed: purple stained fur of perianal region or perineum; and/or discolorations of livers.

TABLE 1: INDIVIDUAL BODY WEIGHT AND DERMAL REACTION DATA

PRIMARY DERMAL IRRITATION STUDY IN RABBITS

TEST ARTICLE: DISPERSE RED 11 - LOT 1

Animal Number	Sex	Initial Body Weight (kg)	24 (±2) Hours** Intact Sites Right ER	Intact Sites Right Side ED	Intact Sites Left ER	Intact Sites Left Side ED	Abraded Sites Right ER	Abraded Sites Right Side ED	Abraded Sites Left ER	Abraded Sites Left Side ED	72 (±2) Hours** Intact Sites Right ER	Intact Sites Right Side ED	Intact Sites Left ER	Intact Sites Left Side ED	Abraded Sites Right ER	Abraded Sites Right Side ED	Abraded Sites Left ER	Abraded Sites Left Side ED
BB9356	M	2.38	3+	2	3+	2	3+	2	3+	2	1d+	1	0+	0	1+	0	0+	0
BB9366	M	2.30	3+	2	2+	2	3+	2	2+	2	1+	0	1d+	0	1+	0	1d+	0
BB9368	M	2.38	2+	2	2+	2	3+	2	3+	2	0+	0	0+	0	1d+	1	1d+	0
BB9369	F	2.36	2+	2	3+	3	3+	3	2+	2	0+	0	1+	0	1+	0	0+	0
BB9394	F	2.12	2+	2	3+	2	3+	2	3+	2	0+	0	0+	0	2d+	1	1+	0
BB9400	F	2.00	2+	2	3+	2	3+	3	2+	2	0+	0	0+	0	1d+	0	0+	0
Total =			14	12	16	13	18	14	15	12	2	1	2	0	7	2	3	0
Average =			(A) 2.3	(B) 2.0	(C) 2.7	(D) 2.2	(E) 3.0	(F) 2.3	(G) 2.5	(H) 2.0	(I) 0.3	(J) 0.2	(K) 0.3	(L) 0.0	(M) 1.2	(N) 0.3	(O) 0.5	(P) 0.0

** = After test article application
ER = Erythema
ED = Edema
+ = Purple discoloration at test site
d = Desquamation

$$\text{Primary Irritation Score} = \frac{A + B + C + D + E + F + G + H + I + J + K + L + M + N + O + P}{8} = 2.7$$

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TABLE 1 (continued): INDIVIDUAL BODY WEIGHT AND DERMAL REACTION DATA

PRIMARY DERMAL IRRITATION STUDY IN RABBITS

TEST ARTICLE: DISPERSE RED 11 - LOT 1

Animal Number	Day 4**								Day 5**							
	Intact Sites				Abraded Sites				Intact Sites				Abraded Sites			
	Right Side		Left Side		Right Side		Left Side		Right Side		Left Side		Right Side		Left Side	
	ER	ED	ER	ED	ER	ED	ER	ED	ER	ED	ER	ED	ER	ED	ER	ED
BB9358	0[d+]	0	0[+]	0	0[+]	0	0[+]	0	0[+]	0	0[+]	0	0[+]	0	0[+]	0
BB9366	0[+]	0	0[d+]	0	0[+]	0	0[d+]	0	0[+]	0	0[+]	0	0[+]	0	0[+]	0
BB9368	0[+]	0	0[+]	0	1[d+]	0	1[d+]	0	0[+]	0	0[+]	0	0[+]	0	0[+]	0
BB9389	0[+]	0	1[+]	0	0[+]	0	0[+]	0	0[+]	0	0[+]	0	0[+]	0	0[+]	0
BB9394	0[+]	0	0[+]	0	1[d+]	1	0[+]	0	0[+]	0	0[+]	0	1[d+]	0	0[+]	0
BB9400	0[+]	0	0[+]	0	0[d+]	0	0[+]	0	0[+]	0	0[+]	0	0[+]	0	0[+]	0

** = After test article application
ER = Erythema
ED = Edema
d = Desquamation
+ = Purple discoloration at test site

TABLE 1 (continued): INDIVIDUAL BODY WEIGHT AND DERMAL REACTION DATA

PRIMARY DERMAL IRRITATION STUDY IN RABBITS

TEST ARTICLE: DISPERSE RED 11 - LOT 1

Animal Number	Day 6**								Day 7**							
	Intact Sites				Abraded Sites				Intact Sites				Abraded Sites			
	Right Side		Left Side		Right Side		Left Side		Right Side		Left Side		Right Side		Left Side	
	ER	ED	ER	ED	ER	ED	ER	ED	ER	ED	ER	ED	ER	ED	ER	ED
BB9356	0[d+]	0	0[+]	0	0[+]	0	0[+]	0	0[+]	0	0[+]	0	0[+]	0	0[+]	0
BB9366	0[+]	0	0[d+]	0	0[+]	0	0[+]	0	0[+]	0	0[+]	0	0[+]	0	0[+]	0
BB9368	0[+]	0	0[+]	0	0[+]	0	0[+]	0	0[+]	0	0[+]	0	0[+]	0	0[+]	0
BB9369	0[+]	0	0[+]	0	0[+]	0	0[+]	0	0[+]	0	0[+]	0	0[+]	0	0[+]	0
BB9394	0[+]	0	0[+]	0	1[d+]	0	0[+]	0	0[+]	0	0[+]	0	0[d+]	0	0[+]	0
BB9400	0[+]	0	0[+]	0	0[+]	0	0[+]	0	0[+]	0	0[+]	0	0[+]	0	0[+]	0

** = After test article application
ER = Erythema
ED = Edema
d = Desquamation
+ = Purple discoloration (stains) at test site

TABLE 2: INDIVIDUAL BODY WEIGHT AND DERMAL REACTION DATA

PRIMARY DERMAL IRRITATION STUDY IN RABBITS

TEST ARTICLE: DISPERSE RED 11 - LOT 2

Animal Number	Sex	Initial Body Weight (kg)	24 (±2) Hours**								72 (±2) Hours**							
			Intact Sites				Abraded Sites				Intact Sites				Abraded Sites			
			Right Side ER	ED	Left Side ER	ED	Right Side ER	ED	Left Side ER	ED	Right Side ER	ED	Left Side ER	ED	Right Side ER	ED	Left Side ER	ED
BB9352	M	2.42	0	0	0	0	1	0	0	0	0	0	0	0	0[d]	0	0	0
BB9354	M	2.32	0	0	1	1	1	0	1	1	0	0	1	0	0	0	0	0
BB9356	M	2.00	0	0	0	0	1	0	1	1	0	0	0	0	0	0	0	0
BB9382	F	2.24	1	0	1	0	1	0	1	1	0	0	0	0	0	0	0	0
BB9385	F	2.28	2	1	1	0	1	0	1	1	1	0	0	0	0	0	1	0
BB9386	F	2.38	1	0	0	0	1	1	2	2	1	0	0	0	1	0	1	0
Total =			4	1	3	1	6	1	6	6	2	0	1	0	1	0	2	0
Average =			(A) 0.7	(B) 0.2	(C) 0.5	(D) 0.2	(E) 1.0	(F) 0.2	(G) 1.0	(H) 1.0	(I) 0.3	(J) 0.0	(K) 0.2	(L) 0.0	(M) 0.2	(N) 0.0	(O) 0.3	(P) 0.0

NOTE: All test sites at the 24 and 72 hour evaluations were stained light purple.
** = After test article application
ER = Erythema
ED = Edema
d = Desquamation

$$\text{Primary Irritation Score} = \frac{A + B + C + D + E + F + G + H + I + J + K + L + M + N + O + P}{8} = 0.73$$

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TABLE 2 (continued): INDIVIDUAL BODY WEIGHT AND DERMAL REACTION DATA

PRIMARY DERMAL IRRITATION STUDY IN RABBITS

TEST ARTICLE: DISPERSE RED 11 - LOT 2

	Day 4**							
	Intact Sites				Abraded Sites			
Animal Number	Right ER	Side ED	Left ER	Side ED	Right ER	Side ED	Left ER	Side ED
BB9352	0[d]	0	0	0	0	0	0	0
BB9354	0	0	0	0	0	0	0	0
BB9356	0	0	0	0	0	0	0	0
BB9382	0	0	0	0	0	0	0	0
BB9385	0	0	0	0	0	0	0	0
BB9386	0	0	0	0	0	0	0	0

NOTE: All test sites at the day 4 evaluation interval were stained light purple.
** = After test article application
ER = Erythema
ED = Edema
d = Desquamation

TABLE 3: INDIVIDUAL BODY WEIGHT AND DERMAL REACTION DATA

PRIMARY DERMAL IRRITATION STUDY IN RABBITS

TEST ARTICLE: DISPERSE BLUE 3

Animal Number	Sex	Initial Body Weight (kg)	24 (±2) Hours** Intact Sites Right Side ER	ED	Left Side ER	ED	Abraded Sites Right Side ER	ED	Left Side ER	ED	72 (±2) Hours** Intact Sites Right Side ER	ED	Left Side ER	ED	Abraded Sites Right Side ER	ED	Left Side ER	ED
BB9405	M	2.34	1[B]	0	1[B]	0	1[B]	0	1[B]	0	0[B]	0	0[B]	0	0[B]	0	0[B]	0
BB9406	M	2.56	2[B]	1	1[B]	0	1[B]	0	1[B]	0	0[B]	0	0[B]	0	0[B]	0	0[B]	0
BB9407	M	2.28	1[B]	0	2[B]	1	1[B]	0	1[B]	0	0[B]	0	0[B]	0	0[B]	0	0[B]	0
BB9434	F	2.48	0[B]	0	0[B]	0	0[B]	0	0[B]	0	0[B]	0	0[B]	0	0[B]	0	0[B]	0
BB9435	F	1.94	1[B]	0	1[B]	1	1[B]	0	0[B]	0	0[B]	0	0[B]	0	0[B]	0	0[B]	0
BB9436	F	2.10	0[B]	0	0[B]	0	0[B]	0	2[B]	0	0[B]	0	0[B]	0	0[B]	0	0[B]	0
Total =			5	1	5	2	4	0	6	1	0	0	0	0	0	0	0	0
Average =			(A) 0.8	(B) 0.2	(C) 0.8	(D) 0.3	(E) 0.7	(F) 0.0	(G) 1.0	(H) 0.2	(I) 0.0	(J) 0.0	(K) 0.0	(L) 0.0	(M) 0.0	(N) 0.0	(O) 0.0	(P) 0.0

** = After test article application
ER = Erythema
ED = Edema
B = Test site discolored blue

Primary Irritation Score = $\frac{A + B + C + D + E + F + G + H + I + J + K + L + M + N + O + P}{8}$ = 0.5

TABLE 4: INDIVIDUAL BODY WEIGHT AND DERMAL REACTION DATA

PRIMARY DERMAL IRRITATION STUDY IN RABBITS

TEST ARTICLE: VIOLET MIXTURE

Animal Number	Sex	Initial Body Weight (kg)	24 (±2) Hours** Intact Sites Right ER	Intact Sites Right Side ED	Intact Sites Left ER	Intact Sites Left Side ED	Abraded Sites Right ER	Abraded Sites Right Side ED	Abraded Sites Left ER	Abraded Sites Left Side ED	72 (±2) Hours** Intact Sites Right ER	Intact Sites Right Side ED	Intact Sites Left ER	Intact Sites Left Side ED	Abraded Sites Right ER	Abraded Sites Right Side ED	Abraded Sites Left ER	Abraded Sites Left Side ED
BB9585	M	2.68	1P	0	0P	0	1P	0	2P	1	0P	0	0P	0	0P	0	0P	0
BB9586	M	2.42	1P	0	1P	0	2P	1	2P	1	0P	0	0P	0	0P	0	1P	0
BB9587	M	2.20	1P	0	0P	0	1P	0	1P	0	0P	0	0P	0	0P	0	0P	0
BB9630	F	3.00	2P	1	2P	1	3P	2	2P	1	1P	0	1P	0	2P	0	1P	0
BB9631	F	2.40	1P	0	1P	0	2P	1	2P	1	0P	0	0P	0	1P	0	1P	0
BB9632	F	2.46	2P	1	1P	1	2P	2	3P	2	0P	0	0P	0	1P	0	1P	0
Total =			8	2	5	2	11	6	12	6	1	0	1	0	4	0	4	0
Average =			(A) 1.3	(B) 0.3	(C) 0.8	(D) 0.3	(E) 1.8	(F) 1.0	(G) 2.0	(H) 1.0	(I) 0.2	(J) 0.0	(K) 0.2	(L) 0.0	(M) 0.7	(N) 0.0	(O) 0.7	(P) 0.0

** = After test article application
ER = Erythema
ED = Edema
P = Test site stained purple

$$\text{Primary Irritation Score} = \frac{A + B + C + D + E + F + G + H + I + J + K + L + M + N + O + P}{8} = 1.3$$

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TABLE 4 (continued): INDIVIDUAL BODY WEIGHT AND DERMAL REACTION DATA

PRIMARY DERMAL IRRITATION STUDY IN RABBITS

TEST ARTICLE: VIOLET MIXTURE

	Day 4**							
	Intact Sites				Abraded Sites			
Animal Number	Right ER	Side ED	Left ER	Side ED	Right ER	Side ED	Left ER	Side ED
BB9585	0[P]	0	0[P]	0	0[P]	0	0[P]	0
BB9586	0[P]	0	0[P]	0	0[P]	0	0[P]	0
BB9587	0[P]	0	0[P]	0	0[P]	0	0[P]	0
BB9630	0[P]	0	0[P]	0	0[P]	0	0[P]	0
BB9631	0[P]	0	0[P]	0	0[P]	0	0[P]	0
BB9632	0[P]	0	0[P]	0	0[P]	0	0[P]	0

** = After test article application
ER = Erythema
ED = Edema
P = Test site stained purple

TABLE 5: INDIVIDUAL BODY WEIGHT AND DERMAL REACTION DATA

PRIMARY DERMAL IRRITATION STUDY IN RABBITS

TEST ARTICLE: SOLVENT RED 1

Animal Number	Sex	Initial Body Weight (kg)	24 (±2) Hours**								72 (±2) Hours**							
			Intact Sites				Abraded Sites				Intact Sites				Abraded Sites			
			Right ER	Side ED	Left ER	Side ED	Right ER	Side ED	Left ER	Side ED	Right ER	Side ED	Left ER	Side ED	Right ER	Side ED	Left ER	Side ED
BB9374	M	2.50	0[R]	0	0[R]	0	0[R]	0	0[R]	0	0[R]	0	0[R]	0	0[R]	0	0[R]	0
BB9375	M	2.66	0[R]	0	0[R]	0	0[R]	0	0[R]	0	0[R]	0	0[R]	0	0[R]	0	0[R]	0
BB9422	M	2.86	0[R]	0	0[R]	0	0[R]	0	0[R]	0	0[R]	0	0[R]	0	0[R]	0	0[R]	0
BB9403	F	3.10	0[R]	0	0[R]	0	0[R]	0	0[R]	0	0[R]	0	0[R]	0	0[R]	0	0[P]	0
BB9439	F	2.74	0[R]	0	0[R]	0	0[R]	0	0[R]	0	0[R]	0	0[R]	0	0[P]	0	0[R]	0
BB9449	F	2.60	0[R]	0	0[R]	0	0[R]	0	0[R]	0	0[R]	0	0[R]	0	0[R]	0	0[R]	0
Total =			0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Average =			(A) 0.0	(B) 0.0	(C) 0.0	(D) 0.0	(E) 0.0	(F) 0.0	(G) 0.0	(H) 0.0	(I) 0.0	(J) 0.0	(K) 0.0	(L) 0.0	(M) 0.0	(N) 0.0	(O) 0.0	(P) 0.0

** = After test article application
ER = Erythema
ED = Edema
P = Test site stained red by test article

$$\text{Primary Irritation Score} = \frac{A + B + C + D + E + F + G + H + I + J + K + L + M + N + O + P}{8} = 0.0$$

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TABLE 6: INDIVIDUAL BODY WEIGHT AND DERMAL REACTION DATA

PRIMARY DERMAL IRRITATION STUDY IN RABBITS

TEST ARTICLE: RED MIXTURE

Animal Number	Sex	Initial Body Weight (kg)	24 (±2) Hours**								72 (±2) Hours**							
			Intact Sites				Abraded Sites				Intact Sites				Abraded Sites			
			Right ER	Side ED	Left ER	Side ED	Right ER	Side ED	Left ER	Side ED	Right ER	Side ED	Left ER	Side ED	Right ER	Side ED	Left ER	Side ED
BB9588	M	2.64	0[R]	0	0[R]	0	0[R]	0	0[R]	0	0[R]	0	0[R]	0	0[R]	0	0[R]	0
BB9589	M	2.72	0[R]	0	0[R]	0	0[R]	0	0[R]	0	0[R]	0	0[R]	0	0[R]	0	0[R]	0
BB9590	M	2.74	0[R]	0	0[R]	0	0[R]	0	0[R]	0	0[R]	0	0[R]	0	0[R]	0	0[R]	0
BB9651	F	2.68	0[R]	0	0[R]	0	0[R]	0	0[R]	0	0[R]	0	0[R]	0	0[R]	0	0[R]	0
BB9652	F	2.52	0[R]	0	0[R]	0	0[R]	0	0[R]	0	0[R]	0	0[R]	0	0[R]	0	0[R]	0
BB9655	F	2.50	0[R]	0	0[R]	0	0[R]	0	0[R]	0	0[R]	0	0[R]	0	0[R]	0	0[R]	0
Total =			0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Average =			(A) 0.0	(B) 0.0	(C) 0.0	(D) 0.0	(E) 0.0	(F) 0.0	(G) 0.0	(H) 0.0	(I) 0.0	(J) 0.0	(K) 0.0	(L) 0.0	(M) 2.0	(N) 0.0	(O) 0.0	(P) 0.0

** = After test article application
ER = Erythema
ED = Edema
R = Test site stained red

$$\text{Primary Irritation Score} = \frac{A + B + C + D + E + F + G + H + I + J + K + L + M + N + O + P}{8} = 0.0$$

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TABLE 7: INDIVIDUAL BODY WEIGHT AND OCULAR REACTION DATA

PRIMARY EYE IRRITATION STUDY IN RABBITS

TEST ARTICLE: DISPERSE RED 11 - LOT 1

Time Interval	Prior to Instillation			24 (±2) Hours			48 (±2) Hours			72 (±2) Hours		
Animal Number	BB 9408	BB 9409	BB 9411	BB 9408	BB 9409	BB 9411	BB 9408	BB 9409	BB 9411	BB 9408	BB 9409	BB 9411
Body Weight (kg)	2.24	2.36	2.44									
I. Cornea												
A. Density	0	0	0	0	0	0	0	0	0	0	0	0
B. Area	0	0	0	0	0	0	0	0	0	0	0	0
A x B x 5	0	0	0	0	0	0	0	0	0	0	0	0
II. Iris												
A. Values	0	0	0	0	0	0	0	0	0	0	0	0
A x 5	0	0	0	0	0	0	0	0	0	0	0	0
III. Conjunctivae												
A. Redness	0	0	0	0+	0+	0+	0+	0+	0+	0+	0+	0+
B. Chemosis	0	0	0	0	0	0	0	0	0	0	0	0
C. Discharge	0	0	0	0	0	0	0	0	0	0	0	0
(A + B + C) x 2	0	0	0	0	0	0	0	0	0	0	0	0
Total I + II + III	0	0	0	0	0	0	0	0	0	0	0	0
Reaction to Fluorescein**	0	0	0	0	0	0	0	0	0	0	0	0
Mean		0.0			0.0			0.0			0.0	
S.D.		0.0			0.0			0.0			0.0	
S.E.		0.0			0.0			0.0			0.0	

** Any fluorescein stain retention in the eye was considered to be epithelial swelling/erosion and not a true stromal opacity. Values were not included in the totals and means.

+ = Test article stain around outside of eye

TABLE 8: INDIVIDUAL BODY WEIGHT AND OCULAR REACTION DATA

PRIMARY EYE IRRITATION STUDY IN RABBITS

TEST ARTICLE: DISPERSE RED 11 - LOT 2

Time Interval	Prior to Instillation			24 (±2) Hours			48 (±2) Hours			72 (±2) Hours		
Animal	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB
Number	9437	9438	9440	9437	9438	9440	9437	9438	9440	9437	9438	9440
Body Weight (kg)	2.38	2.34	2.20									
I. Cornea												
A. Density	0	0	0	0	0	0	0	0	0	0	0	0
B. Area	0	0	0	0	0	0	0	0	0	0	0	0
A x B x 5	0	0	0	0	0	0	0	0	0	0	0	0
II. Iris												
A. Values	0	0	0	0	0	0	0	0	0	0	0	0
A x 5	0	0	0	0	0	0	0	0	0	0	0	0
III. Conjunctivae												
A. Redness	0	0	0	1+	1	1	0	0	0	0	0	0
B. Chemosis	0	0	0	0	1	0	0	0	0	0	0	0
C. Discharge	0	0	0	0	0	0	0	0	0	0	0	0
(A + B + C) x 2	0	0	0	2	4	2	0	0	0	0	0	0
Total I + II + III	0	0	0	2	4	2	0	0	0	0	0	0
Reaction to Fluorescein**	0	0	0	0	0	0	0	0	0	0	0	0
Mean		0.0			2.7			0.0			0.0	
S.D.		0.0			1.2			0.0			0.0	
S.E.		0.0			0.7			0.0			0.0	

** Any fluorescein stain retention in the eye was considered to be epithelial swelling/erosion and not a true stromal opacity. Values were not included in the totals and means.

+ = Test article remaining under lower lid of test eye.

NOTE: All test eyes at the 24, 48, and 72 hour evaluations were stained purple around the eyelids.

TABLE 9: INDIVIDUAL BODY WEIGHT AND OCULAR REACTION DATA

PRIMARY EYE IRRITATION STUDY IN RABBITS

TEST ARTICLE: DISPERSE BLUE 3

Time Interval	Prior to Instillation			24 (±2) Hours			48 (±2) Hours			72 (±2) Hours		
Animal Number	BB 9441	BB 9443	BB 9445	BB 9441	BB 9443	BB 9445	BB 9441	BB 9443	BB 9445	BB 9441	BB 9443	BB 9445
Body Weight (kg)	2.38	2.06	2.52									
I. Cornea												
A. Density	0	0	0	0	0	0	0	0	0	0	0	0
B. Area	0	0	0	0	0	0	0	0	0	0	0	0
A x B x 5	0	0	0	0	0	0	0	0	0	0	0	0
II. Iris												
A. Values	0	0	0	0	0	0	0	0	0	0	0	0
A x 5	0	0	0	0	0	0	0	0	0	0	0	0
III. Conjunctivae												
A. Redness	0	0	0	0[b]	0[b]	0[b]	0[b]	0[b]	0[b]	0[b]	0[b]	0[b]
B. Chemosis	0	0	0	0	0	0	0	0	0	0	0	0
C. Discharge	0	0	0	0	0	0	0	0	0	0	0	0
(A + B + C) x 2	0	0	0	0	0	0	0	0	0	0	0	0
Total I + II + III	0	0	0	0	0	0	0	0	0	0	0	0
Reaction to Fluorescein**	0	0	0	0	0	0	0	0	0	0	0	0
Mean		0.0			0.0			0.0			0.0	
S.D.		0.0			0.0			0.0			0.0	
S.E.		0.0			0.0			0.0			0.0	

** Any fluorescein stain retention in the eye was considered to be epithelial swelling/erosion and not a true stromal opacity. Values were not included in the totals and means.
b = Right side of head stained blue

TABLE 10: INDIVIDUAL BODY WEIGHT AND OCULAR REACTION DATA

PRIMARY EYE IRRITATION STUDY IN RABBITS

TEST ARTICLE: VIOLET MIXTURE

Time Interval	Prior to Instillation			24 (±2) Hours			48 (±2) Hours			72 (±2) Hours		
Animal Number	BB 9633	BB 9634	BB 9636	BB 9633	BB 9644	BB 9636	BB 9633	BB 9634	BB 9636	BB 9633	BB 9634	BB 9636
Body Weight (kg)	2.10	2.00	2.14									
I. Cornea												
A. Density	0	0	0	0	0	0	0	0	0	0	0	0
B. Area	0	0	0	0	0	0	0	0	0	0	0	0
A x B x 5	0	0	0	0	0	0	0	0	0	0	0	0
II. Iris												
A. Values	0	0	0	0	0	0	0	0	0	0	0	0
A x 5	0	0	0	0	0	0	0	0	0	0	0	0
III. Conjunctivae												
A. Redness	0	0	0	1[P]	0[P]	1[P]	0[P]	0[P]	0[P]	0	0	0
B. Chemosis	0	0	0	1	0	1	0	0	0	0	0	0
C. Discharge	0	0	0	0	0	0	0	0	0	0	0	0
(A + B + C) x 2	0	0	0	4	0	4	0	0	0	0	0	0
Total I + II + III	0	0	0	4	0	4	0	0	0	0	0	0
Reaction to Fluorescein**	0	0	0	0	0	0	0	0	0	0	0	0
Mean		0.0			2.7			0.0			0.0	
S.D.		0.0			2.3			0.0			0.0	
S.E.		0.0			1.3			0.0			0.0	

** Any fluorescein stain retention in the eye was considered to be epithelial swelling/erosion and not a true stromal opacity. Values were not included in the totals and means.

+ = Test article stain around outside of eye

P = Purple stained fur around treated eye and on all feet

TABLE II: INDIVIDUAL BODY WEIGHT AND OCULAR REACTION DATA

PRIMARY EYE IRRITATION STUDY IN RABBITS

TEST ARTICLE: SOLVENT RED 1

Time Interval	Prior to Instillation			24 (±2) Hours			48 (±2) Hours			72 (±2) Hours		
Animal Number	BB 9526	BB 9545	BB 9552	BB 9526	BB 9545	BB 9552	BB 9526	BB 9545	BB 9552	BB 9526	BB 9545	BB 9552
Body Weight (kg)	2.48	2.52	2.58									
I. Cornea												
A. Density	0	0	0	1	0	1	0	0	0	0	0	0
B. Area	0	0	0	1	0	1	0	0	0	0	0	0
A x B x 5	0	0	0	5	0	5	0	0	0	0	0	0
II. Iris												
A. Values	0	0	0	1	0	1	0	0	0	0	0	0
A x 5	0	0	0	5	0	5	0	0	0	0	0	0
III. Conjunctivae												
A. Redness	0	0	0	$3^{b,t,}_{r}$	$2^{b,t,}_{r}$	$2^{b,t,}_{r}$	2^{r}	$1^{t,r}$	1^{r}	1^{r}	0^{r}	0^{r}
B. Chemosis	0	0	0	2	2	2	1	0	1	0	0	0
C. Discharge	0	0	0	2	1	2	0	0	0	0	0	0
(A + B + C) x 2	0	0	0	14	10	12	6	2	4	2	0	0
Total I + II + III	0	0	0	24	10	22	6	2	4	2	0	0
Reaction to Fluorescein**	0	0	0	1	0	1	0	0	0	0	0	0
Mean		0.0			10.7			4.0			0.7	
S.D.		0.0			7.5			2.0			1.2	
S.E.		0.0			4.4			1.2			0.7	

** Any fluorescein stain retention in the eye was considered to be epithelial swelling/erosion and not a true stromal opacity. Values were not included in the totals and means.

b = Blistering of the conjucntivae

t = Test article present in eye

r = Right side of head stained red

TABLE 11 (continued): INDIVIDUAL BODY WEIGHT AND OCULAR REACTION DATA

PRIMARY EYE IRRITATION STUDY IN RABBITS

TEST ARTICLE: SOLVENT RED 1

Animal Number	Day 7 BB 9526	BB 9545	BB 9552
I. Cornea			
A. Density	0	0	0
B. Area	0	0	0
A x B x 5	0	0	0
II. Iris			
A. Values	0	0	0
A x 5	0	0	0
III. Conjunctivae			
A. Redness	0	0[r]	0[r]
B. Chemosis	0	0	0
C. Discharge	0	0	0
(A + B + C) x 2	0	0	0
Total I + II + III	0	0	0
Reaction to Fluorescein**	0	0	0
Mean		0.0	
S.D.		0.0	
S.E.		0.0	

** Any fluorescein stain retention in the eye was considered to be epithelial swelling/erosion and not a true stromal opacity. Values were not included in the totals and means.
r = Right side of head stained red

TABLE 12: INDIVIDUAL BODY WEIGHT AND OCULAR REACTION DATA

PRIMARY EYE IRRITATION STUDY IN RABBITS

TEST ARTICLE: RED MIXTURE

Time Interval	Prior to Instillation			24 (±2) Hours			48 (±2) Hours			72 (±2) Hours		
Animal	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB
Number	9591	9592	9594	9591	9592	9594	9591	9592	9594	9591	9592	9594
Body Weight (kg)	2.28	2.64	2.68									
I. Cornea												
A. Density	0	0	0	1	1	1	1	0	0	1	0	0
B. Area	0	0	0	1	1	1	1	0	0	1	0	0
A x B x 5	0	0	0	5	5	5	5	0	0	5	0	0
II. Iris												
A. Values	0	0	0	1	1	1	1	0	0	1	0	0
A x 5	0	0	0	5	5	5	5	0	0	5	0	0
III. Conjunctivae												
A. Redness	0	0	0	$2^{b,t,r}$	$3^{b,t,r}$	$2^{b,t,r}$	3^{r}	2^{r}	3^{r}	3^{r}	1^{r}	2^{r}
B. Chemosis	0	0	0	3	2	3	2	1	2	2	1	1
C. Discharge	0	0	0	3	3	3	2	0	0	2	0	0
(A + B + C) x 2	0	0	0	16	16	16	14	6	10	14	4	6
Total I + II + III	0	0	0	26	26	26	24	6	10	24	4	6
Reaction to Fluorescein**	0	0	0	1	1	1	1	0	0	0	0	0
Mean		0.0			26.0			12.3			11.3	
S.D.		0.0			0.0			9.5			11.0	
S.E.		0.0			0.0			5.5			6.4	

** Any fluorescein stain retention in the eye was considered to be epithelial swelling/erosion and not a true stromal opacity. Values were not included in the totals and means.
b = Blistering of the conjunctivae
t = Test article present in eye
r = Right side of head stained red

TABLE 12 (continued): INDIVIDUAL BODY WEIGHT AND OCULAR REACTION DATA

PRIMARY EYE IRRITATION STUDY IN RABBITS

TEST ARTICLE: RED MIXTURE

Time Interval	Day 7			Day 14			Day 21		
Animal Number	BB 9591	BB 9592	BB 9594	BB 9591	BB 9592	BB 9594	BB 9591	BB 9592	BB 9594
I. Cornea									
A. Density	1	0	0	1	0	0	1	0	0
B. Area	1	0	0	1	0	0	1	0	0
A x B x 5	5	0	0	5	0	0	5	0	0
II. Iris									
A. Values	1	0	0	0	0	0	0	0	0
A x 5	5	0	0	0	0	0	0	0	0
III. Conjunctivae									
A. Redness	2[r,n]	0[r]	0[r]	1[r,n]	0[r]	0[r]	0[r,n]	0[r]	0[r]
B. Chemosis	2	0	0	2	0	0	0	0	0
C. Discharge	3	0	0	1	0	0	0	0	0
(A + B + C) x 2	14	0	0	8	0	0	0	0	0
Total I + II + III	24	0	0	13	0	0	5	0	0
Reaction to Fluorescein**	1	0	0	0	0	0	0	0	0
Mean		8.0			4.3			1.7	
S.D.		13.9			7.5			2.9	
S.E.		8.0			4.3			1.7	

** Any fluorescein stain retention in the eye was considered to be epithelial swelling/erosion and not a true stromal opacity. Values were not included in the totals and means.
r = Right side of head stained red
n = Nicks in upper and lower lids

TABLE 13

INDIVIDUAL BODY WEIGHT AND TEST ARTICLE ADMINISTRATION DATA

ACUTE DERMAL TOXICITY STUDY IN RABBITS

TEST ARTICLE: DISPERSE RED 11 - LOT 1

Animal Number	Sex	Body Weight (kilograms) Day of Study 0	3	7	10	14	Total Weight Change (kg)	Amount of Test Article Administered (mg)
BB9413	M	2.72	2.70	2.66	2.66	2.64	-0.08	5,440
BB9414	M	2.26	2.30	2.40	2.50	2.68	0.42	4,520
BB9415	M	2.80	2.76	2.88	2.96	3.02	0.22	5,600
BB9416	M	2.68	2.74	2.88	3.02	3.16	0.48	5,360
BB9417	M	2.50	2.48	2.54	2.58	2.66	0.16	5,000
Mean		2.59	2.60	2.67	2.74	2.83	0.24	5,184
S.D.		0.22	0.20	0.21	0.23	0.24	0.22	431
S.E.		0.10	0.09	0.09	0.10	0.11	0.10	193
BB9442	F	2.34	2.38	2.44	2.48	2.68	0.34	4,680
BB9432	F	2.06	2.02	2.10	2.06	2.16	0.10	4,120
BB9433	F	2.32	2.32	2.48	2.54	2.66	0.34	4,640
BB9444	F	2.64	2.66	2.76	2.86	2.80	0.16	5,280
BB9446	F	2.34	2.32	2.52	2.56	2.52	0.18	4,680
Mean		2.34	2.34	2.46	2.50	2.56	0.22	4,680
S.D.		0.21	0.23	0.24	0.29	0.25	0.11	411
S.E.		0.09	0.10	0.11	0.13	0.11	0.05	184

TABLE 14

INDIVIDUAL ANTEMORTEM OBSERVATIONS

ACUTE DERMAL TOXICITY STUDY IN RABBITS

TEST ARTICLE: DISPERSE RED 11 - LOT I

		Day(s) Finding Observed									
Finding	Animal No.: Sex:	BB9413 (M)	BB9414 (M)	BB9415 (M)	BB9416 (M)	BB9417 (M)	BB9442 (F)	BB9432 (F)	BB9433 (F)	BB9444 (F)	BB9446 (F)
Test site, feet, and/or muzzle discolored purple		1-14	1-14	1-14	1-14	1-14	1-14	1-14	1-14	1-14	1-14
Erythema and/or edema			1-4		1			1		1,2	
Purple colored urine			3,4	3-5	3,4	3-6	3-5	3,4	3-6	3-6	3,4
Loose stools		7,8									
Yellow/brown stained fur - perianal region		7-14									
No abnormalities		0	0	0	0	0	0	0	0	0	0

TABLE 15

INDIVIDUAL NECROPSY FINDINGS

ACUTE DERMAL TOXICITY STUDY IN RABBITS

TEST ARTICLE: DISPERSE RED 11 - LOT 1

Animal Number	Sex	Abnormalities Noted at Necropsy (Organ - Abnormality)
BB9413	M	Treated Skin - Discoloration, purple, scattered areas External Surface - Discoloration, purple, fur of feet
BB9414	M	Treated Skin - Discoloration, purple, scattered areas External Surface - Discoloration, purple, fur of feet
BB9415	M	Treated Skin - Discoloration, purple, scattered areas External Surface - Discoloration, purple, skin of feet
BB9416	M	Treated Skin - Discoloration, purple, scattered areas External Surface - Discoloration, purple, fur of feet
BB9417	M	Treated Skin - Discoloration, purple, scattered areas External Surface - Discoloration, purple, fur of feet, abdomen, and face
BB9442	F	Treated Skin - Discoloration, red, linear, anterior portion of treated area Discoloration, purple, scattered areas External Surface - Discoloration, purple, fur of feet
BB9432	F	Treated Skin - Discoloration, purple, scattered areas External Surface - Discoloration, purple, fur of feet
BB9433	F	Treated Skin - Discoloration, purple, scattered areas External Surface - Discoloration, purple, fur of feet

TABLE 15 (continued)

INDIVIDUAL NECROPSY FINDINGS

ACUTE DERMAL TOXICITY STUDY IN RABBITS

TEST ARTICLE: DISPERSE RED 11 - LOT 1

Animal Number	Sex	Abnormalities Noted at Necropsy (Organ - Abnormality)
BB9444	F	Treated Skin - Discoloration, purple, scattered areas External Surface - Discoloration, purple, fur of feet
BB9446	F	Treated Skin - Discoloration, purple, scattered areas External Surface - Discoloration, purple, fur of feet

TABLE 16

INDIVIDUAL BODY WEIGHT AND TEST ARTICLE ADMINISTRATION DATA

ACUTE DERMAL TOXICITY STUDY IN RABBITS

TEST ARTICLE: DISPERSE RED 11 - LOT 2

Animal Number	Sex	Body Weight (kilograms) Day of Study 0	3	7	10	14	Total Weight Change (kg)	Amount of Test Article Administered (mg)
BB9424	M	2.34	2.34	2.44	2.44	(2.10)	--	4,680
BB9425	M	2.28	2.38	2.52	2.56	2.48	0.20	4,560
BB9428	M	2.48	2.44	2.46	2.46	2.56	0.08	4,960
BB9429	M	2.54	2.48	2.46	2.36	2.52	-0.02	5,080
BB9430	M	2.38	2.44	2.48	2.46	2.54	0.16	4,760
Mean		2.40	2.42	2.47	2.46	2.53	0.11	4,808
S.D.		0.11	0.05	0.03	0.07	0.03	0.10	211
S.E.		0.05	0.02	0.01	0.03	0.02	0.05	94
BB9447	F	2.50	2.62	2.68	2.72	2.84	0.34	5,000
BB9454	F	2.42	2.46	2.64	2.68	2.74	0.32	4,840
BB9455	F	2.56	2.58	2.60	2.76	2.80	0.24	5,120
BB9456	F	2.70	2.80	2.92	2.98	3.06	0.36	5,400
BB9457	F	2.70	2.66	2.74	2.92	3.04	0.34	5,400
Mean		2.58	2.62	2.72	2.80	2.90	0.32	5,152
S.D.		0.12	0.12	0.13	0.14	0.15	0.05	242
S.E.		0.06	0.06	0.06	0.06	0.06	0.02	111

Numbers in parentheses denote post-mortem body weights.
-- = Not determined

TABLE 17

INDIVIDUAL ANTEMORTEM OBSERVATIONS

ACUTE DERMAL TOXICITY STUDY IN RABBITS

TEST ARTICLE: DISPERSE RED 11 - LOT 2

Finding	Animal No.: / Sex:	BB9424 (M)	BB9425 (M)	BB9428 (M)	BB9429 (M)	BB9430 (M)	BB9447 (F)	BB9454 (F)	BB9455 (F)	BB9456 (F)	BB9457 (F)
		Day(s) Finding Observed									
Test site, feet, and muzzles stained violet		2-13	2-14	2-14	2-14	2-14	2-14	2-14	2-14	2-14	2-14
Violet colored urine		3,4	3,4	3,4	3,4	3,4	3,4	3,4	3,4	3,4	3,4
No abnormalities		0,1	0,1	0,1	0,1	0,1	0,1	0,1	0,1	0,1	0,1
Death		14									

TABLE 18

INDIVIDUAL NECROPSY FINDINGS

ACUTE DERMAL TOXICITY STUDY IN RABBITS

TEST ARTICLE: DISPERSE RED 11 - LOT 2

Animal Number	Sex	Abnormalities Noted at Necropsy (Organ - Abnormality)
BB9424*	M	Treated Skin - Discoloration, purple, scattered areas Lung - Consolidation, diffuse, red, firm Exudate, tan, mild, right lobes External Surface - Discoloration, pink, fur of feet
BB9425	M	Treated Skin - Discoloration, purple, scattered areas External Surface - Discoloration, purple, fur of feet
BB9428	M	Treated Skin - Discoloration, purple, fur of feet External Surface - Discoloration, purple, fur of feet
BB9429	M	External Surface - Discoloration, purple, fur of feet Treated Skin - Discoloration, purple, fur of feet
BB9430	M	Treated Skin - Discoloration, solitary, raised, purple Discoloration, purple, scattered areas External Surface - Discoloration, purple, fur of feet
BB9447	F	Treated Skin - Discoloration, purple, fur of feet Urinary Bladder - Distended with fluid External Surface - Discoloration, purple, fur of feet
BB9454	F	Treated Skin - Discoloration, purple, scattered areas External Surface - Discoloration, purple, fur of feet
BB9455	F	Treated Skin - Discoloration, purple, scattered areas External Surface - Discoloration, purple, fur of feet

* = Animal found dead

TABLE 18 (continued)

INDIVIDUAL NECROPSY FINDINGS

ACUTE DERMAL TOXICITY STUDY IN RABBITS

TEST ARTICLE: DISPERSE RED 11 - LOT 2

Animal Number	Sex	Abnormalities Noted at Necropsy (Organ - Abnormality)
BB9456	F	Treated Skin - Discoloration, purple, scattered areas External Surface - Discoloration, purple, fur of feet
BB9457	F	Treated Skin - Discoloration, purple, scattered areas External Surface - Discoloration, purple, fur of feet

TABLE 19

INDIVIDUAL BODY WEIGHT AND TEST ARTICLE ADMINISTRATION DATA

ACUTE DERMAL TOXICITY STUDY IN RABBITS

TEST ARTICLE: DISPERSE BLUE 3

Animal Number	Sex	Body Weight (kilograms) Day of Study 0	3	7	10	14	Total Weight Change (kg)	Amount of Test Article Administered (mg)
BB9418	M	2.44	2.48	2.44	2.46	2.54	0.10	4,680
BB9412	M	2.32	2.20	2.20	2.38	2.44	0.12	4,640
BB9418	M	2.60	2.60	2.74	2.78	2.86	0.26	5,200
BB9419	M	2.84	2.86	2.94	3.00	3.14	0.30	5,680
BB9423	M	2.56	2.64	2.72	2.82	2.92	0.36	5,120
Mean		2.55	2.56	2.61	2.69	2.78	0.23	5,104
S.D.		0.19	0.24	0.29	0.26	0.29	0.11	389
S.E.		0.09	0.11	0.13	0.12	0.13	0.05	174
BB9445	F	2.60	2.60	2.70	2.58	2.80	0.20	5,200
BB9450	F	2.20	2.20	2.50	2.60	2.74	0.46	4,560
BB9451	F	2.66	2.74	2.92	2.98	3.14	0.48	5,320
BB9452	F	2.66	2.70	2.76	2.76	2.86	0.20	5,320
BB9453	F	2.42	2.46	2.60	2.60	2.68	0.26	4,840
Mean		2.52	2.56	2.70	2.70	2.84	0.32	5,048
S.D.		0.17	0.19	0.16	0.17	0.18	0.14	336
S.E.		0.08	0.08	0.07	0.08	0.08	0.06	150

TABLE 20

INDIVIDUAL ANTEMORTEM OBSERVATIONS

ACUTE DERMAL TOXICITY STUDY IN RABBITS

TEST ARTICLE: DISPERSE BLUE 3

Animal Number	Sex	Finding	Day(s) Finding Observed
BB9410	M	No abnormalities	0
		Test site stained blue	1-14
		Head and feet stained blue	2-14
BB9412	M	No abnormalities	0
		Test site stained blue	1-14
		Head stained blue	2-12
		Feet stained blue	2-14
		Few stools	6
BB9418	M	No abnormalities	0
		Test site stained blue	1-14
		Head stained blue	2-12
		Feet stained blue	2-14
BB9419	M	No abnormalities	0
		Test site stained blue	1-14
		Head stained blue	2-11
		Feet stained blue	2-14
BB9423	M	No abnormalities	0
		Test site stained blue	1-14
		Head stained blue	2-12
		Feet stained blue	2-14
BB9448	F	No abnormalities	0
		Test site stained blue	1-14
		Head and feet stained blue	2-14
BB9450	F	No abnormalities	0
		Test site stained blue	1-14
		Head stained blue	2-11
		Feet stained blue	2-14
BB9451	F	No abnormalities	0
		Test site stained blue	1-14
		Head stained blue	2-11
		Feet stained blue	2-14

TABLE 20 (continued)

INDIVIDUAL ANTEMORTEM OBSERVATIONS

ACUTE DERMAL TOXICITY STUDY IN RABBITS

TEST ARTICLE: DISPERSE BLUE 3

Animal Number	Sex	Finding	Day(s) Finding Observed
BB9452	F	No abnormalities	0
		Test site stained blue	1-14
		Head and feet stained blue	2-14
BB9453	F	No abnormalities	0
		Test site stained blue	1-14
		Head stained blue	2-12
		Feet stained blue	2-14

TABLE 21

INDIVIDUAL NECROPSY FINDINGS

ACUTE DERMAL TOXICITY STUDY IN RABBITS

TEST ARTICLE: DISPERSE BLUE 3

Animal Number	Sex	Abnormalities Noted at Necropsy (Organ - Abnormality)
BB9410	M	Treated Skin - Discoloration, blue, scattered areas External Surface - Pale blue discoloration of fur, feet, and face
BB9412	M	Treated Skin - Discoloration, blue, scattered areas External Surface - Discoloration, pale blue, fur of feet
BB9418	M	Treated Skin - Discoloration, blue, scattered areas External Surface - Discoloration, blue, fur of feet Discoloration, pink, fur of left anterior and right posterior feet*
BB9419	M	Treated Skin - Discoloration, blue, scattered areas External Surface - Discoloration, blue, fur of feet
BB9423	M	Treated Skin - Discoloration, blue, scattered areas External Surface - Discoloration, blue, fur of feet
BB9448	F	Treated Skin - Discoloration, blue, scattered areas External Surface - Discoloration, blue, fur of feet and face
BB9450	F	Treated Skin - Discoloration, blue, scattered areas External Surface - Discoloration, blue, fur of feet
BB9451	F	Treated Skin - Discoloration, blue, scattered areas External Surface - Discoloration, blue, fur of feet
BB9452	F	Treated Skin - Discoloration, blue, scattered areas External Surface - Discoloration, blue, fur around nose and on feet
BB9453	F	Treated Skin - Discoloration, blue, scattered areas External Surface - Discoloration, blue, fur of feet

* This stain was apparently picked up off of the table the animal was placed on to re-clip the test site area prior to taking the animal to pathology and is not related to the test article nor the results of the study per se.

TABLE 22

INDIVIDUAL BODY WEIGHT AND TEST ARTICLE ADMINISTRATION DATA

ACUTE DERMAL TOXICITY STUDY IN RABBITS

TEST ARTICLE: VIOLET MIXTURE

Animal Number	Sex	Body Weight (kilograms) Day of Study 0	3	7	10	14	Total Weight Change (kg)	Amount of Test Article Administered (mg)
BB9605	M	2.58	2.66	2.64	2.72	2.78	0.20	5,200
BB9597	M	2.26	2.34	2.48	2.52	2.58	0.32	4,500
BB9608	M	2.80	2.72	2.80	2.84	2.98	0.18	5,600
BB9609	M	2.56	2.60	2.82	2.80	2.96	0.38	5,200
BB9426	M	2.88	2.94	3.06	3.08	3.10	0.22	5,800
Mean		2.62	2.65	2.76	2.79	2.86	0.24	5,260
S.D.		0.24	0.22	0.22	0.20	0.20	0.11	498
S.E.		0.11	0.10	0.10	0.09	0.09	0.05	223
BB9626	F	2.36	2.30	2.42	2.50	2.66	0.30	4,700
BB9645	F	2.48	2.38	2.40	2.46	2.28	-0.20	5,000
BB9646	F	2.40	2.20	2.38	2.48	2.62	0.22	4,800
BB9649	F	2.38	2.44	2.68	2.52	2.40	0.02	4,800
BB9650	F	2.56	2.44	2.22	1.98	2.18	-0.38	5,100
Mean		2.44	2.35	2.42	2.39	2.43	-0.01	4,880
S.D.		0.08	0.10	0.17	0.23	0.21	0.28	164
S.E.		0.04	0.05	0.07	0.10	0.09	0.13	73

TABLE 23

INDIVIDUAL ANTEMORTEM OBSERVATIONS

ACUTE DERMAL TOXICITY STUDY IN RABBITS

TEST ARTICLE: VIOLET MIXTURE

Finding	Animal No.: Sex:	BB9605 (M)	BB9597 (M)	BB9608 (M)	BB9609 (M)	BB9426 (M)	BB9626 (F)	BB9645 (F)	BB9646 (F)	BB9649 (F)	BB9650 (F)
		Day(s) Finding Observed									
Test site, nose, and/or feet discolored purple		1-14	1-14	1-14	1-14	1-14	1-14	1-14	1-14	1-14	1-14
Purple colored urine		2,3	1-5	2-7	2,3	2,3	1-3	3-8	2-6	2,3	2-7
Loose stools							3				4,5
No urine								2,14		11,12, 14	8,10,11
Few stools								2-4	3	10	11,12
Mucous-like stools								13			
Food appeared undisturbed								13,14		9-14	5-13
Pale											7-10
Emaciated											10-14
Abdominal region appeared bloated											10,11
No stools								14	2	9,11-14	6-10
No abnormalities		0	0	0	0	0	0	0	0	0	0

TABLE 24

INDIVIDUAL NECROPSY FINDINGS

ACUTE DERMAL TOXICITY STUDY IN RABBITS

TEST ARTICLE: VIOLET MIXTURE

Animal Number	Sex	Abnormalities Noted at Necropsy (Organ - Abnormality)
BB9426	M	Treated Skin - Discoloration, diffuse, purple External Surface - Fur of feet purple
BB9597	M	Treated Skin - Discoloration, diffuse, purple External Surface - Fur of feet purple
BB9605	M	Treated Skin - Discoloration, diffuse, purple External Surface - Fur of feet purple
BB9608	M	Treated Skin - Discoloration, diffuse, purple External Surface - Fur of feet purple
BB9609	M	Treated Skin - Discoloration, diffuse, pale purple External Surface - Fur of feet purple Kidney - Depression, multiple, focal, bilateral cortex
BB9626	F	Treated Skin - Discoloration, diffuse, purple External Surface - fur of feet purple
BB9645	F	Treated Skin - Discoloration, diffuse, purple External Surface - Fur of feet purple
BB9646	F	Treated Skin - Discoloration, diffuse, purple External Surface - Fur of feet purple
BB9649	F	Treated Skin - Discoloration, diffuse, purple External Surface - Fur of feet purple Small Intestine - Distended with fluid contents
BB9650	F	Treated Skin - Discoloration, diffuse, purple External Surface - Fur of feet purple Purple fur, perineum Urinary Bladder - Contains purple-tinged fluid

TABLE 25

INDIVIDUAL BODY WEIGHT AND TEST ARTICLE ADMINISTRATION DATA

ACUTE DERMAL TOXICITY STUDY IN RABBITS

TEST ARTICLE: SOLVENT RED 1

Animal No. Lei	Sex	Body Weight (kilograms) Day of Study 0	3	7	10	14	Total Weight Change (kg)	Amount of Test Article Administered (mg)
BB9477	M	2.84	2.80	2.88	2.88	2.92	0.08	5,680
BB9484	M	2.58	2.66	2.74	2.78	2.84	0.26	5,160
BB9487	M	2.14	2.28	2.38	2.46	2.56	0.42	4,280
BB9486	M	2.24	2.32	2.54	2.64	2.78	0.54	4,480
BB9490	M	2.26	2.32	2.42	2.38	2.48	0.22	4,520
Mean		2.41	2.48	2.59	2.63	2.72	0.30	4,824
S.D.		0.29	0.24	0.21	0.21	0.19	0.18	581
S.E.		0.13	0.11	0.10	0.09	0.08	0.08	260
BB9535	F	2.60	2.72	2.82	2.90	3.10	0.50	5,200
BB9536	F	2.38	2.44	2.58	2.64	2.74	0.36	4,760
BB9537	F	2.30	2.26	2.36	2.34	2.42	0.12	4,600
BB9538	F	2.42	2.46	2.68	2.66	2.60	0.18	4,840
BB9556	F	2.28	2.34	2.40	2.46	2.54	0.26	4,560
Mean		2.40	2.44	2.57	2.60	2.68	0.28	4,792
S.D.		0.13	0.17	0.19	0.21	0.26	0.15	255
S.E.		0.06	0.08	0.09	0.10	0.12	0.07	114

TABLE 26

INDIVIDUAL ANTEMORTEM OBSERVATIONS

ACUTE DERMAL TOXICITY STUDY IN RABBITS

TEST ARTICLE: SOLVENT RED 1

Finding	Animal No.: Sex:	BB9477 (M)	BB9484 (M)	BB9487 (M)	BB9488 (M)	BB9490 (M)	BB9535 (F)	BB9536 (F)	BB9537 (F)	BB9538 (F)	BB9556 (F)
		Day(s) Finding Observed									
Test site stained red		1-13	1-14	1-14	1-14	1-14	1-14	1-14	1-14	1-14	1-14
Feet discolored red		2-13	2-14	2-14	2-14	2-14	2-14	2-14	2-14	2-14	2-14
Head discolored red		2-12	2-12	2-12	2-12	2-14	2-12	2-14	2-12	2-14	2-12
Few stools		2									
Loose stool		14									
No abnormalities		0	0	0	0	0	0	0	0	0	0

TABLE 27

INDIVIDUAL NECROPSY FINDINGS

ACUTE DERMAL TOXICITY STUDY IN RABBITS

TEST ARTICLE: SOLVENT RED 1

Animal Number	Sex	Abnormalities Noted at Necropsy (Organ - Abnormality)
BB9477	M	None
BB9484	M	Treated Skin - Discoloration, red, scattered areas Fur of Back and Feet - Discoloration, red
BB9487	M	Treated Skin - Discoloration, red, scattered areas Fur of Back and Feet - Discoloration, red
BB9488	M	Treated Skin - Discoloration, red, scattered areas Fur of Back, Feet, and Abdomen - Discoloration, red
BB9490	M	Treated Skin - Discoloration, red, scattered areas Fur of Back, Neck, Abdomen, and Feet - Discoloration, red Lung - Discoloration, multifocal, red, pinpoint, all lobes
BB9535	F	Treated Skin - Discoloration, dark red, thickened, 2 areas Fur of Back, Feet, and Abdomen - Discoloration, red Liver - Discoloration, solitary, dark red, margin of left lateral lobe
BB9536	F	Treated Skin - Discoloration, red, scattered areas Fur of Back, Feet, and Abdomen - Discoloration, red
BB9537	F	Treated Skin - Discoloration, red, scattered areas Fur of Back and Feet - Discoloration, red
BB9538	F	Treated Skin - Discoloration, red, scattered areas Fur of Back, Feet, Abdomen, and Head - Discoloration, red
BB9556	F	Fur of Back, Feet, and Abdomen - Discoloration, red Lung - Discoloration, multiple focal, red, several lobes

TABLE 28

INDIVIDUAL BODY WEIGHT AND TEST ARTICLE ADMINISTRATION DATA

ACUTE DERMAL TOXICITY STUDY IN RABBITS

TEST ARTICLE: RED MIXTURE

Animal Number	Sex	Body Weight (kilograms) Day of Study 0	3	7	10	14	Total Weight Change (kg)	Amount of Test Article Administered (mg)
BB9789	M	2.50	2.54	2.74	2.82	3.06	0.56	5,000
BB9800	M	2.24	2.26	2.44	2.50	2.62	0.38	4,480
BB9801	M	2.20	2.30	2.44	2.54	2.68	0.48	4,400
BB9802	M	2.34	2.46	2.58	2.60	2.64	0.30	4,680
BB9806	M	2.26	2.28	2.44	2.50	2.64	0.38	4,520
Mean		2.31	2.37	2.53	2.59	2.71	0.40	4,616
S.D.		0.12	0.12	0.13	0.13	0.20	0.10	238
S.E.		0.05	0.06	0.06	0.06	0.09	0.04	106
BB9817	F	2.28	1.96	2.36	2.44	2.62	0.34	4,560
BB9824	F	2.22	2.26	2.38	2.40	2.44	0.22	4,440
BB9831	F	2.24	2.38	2.44	2.50	2.62	0.38	4,480
BB9833	F	2.26	2.32	2.40	2.48	2.58	0.32	4,520
BB9834	F	2.38	2.22	2.22	2.24	2.30	-0.08	4,760
Mean		2.28	2.21	2.36	2.41	2.51	0.24	4,552
S.D.		0.06	0.15	0.08	0.10	0.14	0.19	125
S.E.		0.03	0.07	0.04	0.05	0.06	0.08	56

TABLE 29

INDIVIDUAL ANTEMORTEM OBSERVATIONS

ACUTE DERMAL TOXICITY STUDY IN RABBITS

TEST ARTICLE: RED MIXTURE

Finding	Animal No.: Sex:	BB9789 (M)	BB9800 (M)	BB9801 (M)	BB9802 (M)	BB9806 (M)	BB9817 (F)	BB9824 (F)	BB9831 (F)	BB9833 (F)	BB9834 (F)
		Day(s) Finding Observed									
Test site stained red		1-14	1-14	1-14	1-14	1-14	1-14	1-14	1-14	1-14	1-14
Feet stained red		2-14	2-14	2-14	2-14	2-14	2-14	2-14	2-14	2-14	2-14
Abdomen stained red		2-14	2-14	2-14	2-14	2-14	2-13	2-13	2-14	2-14	2-14
Head stained red		2-6	2-14	2-6	2-14	2-14	2-14	2-14	2-14	2-6	2-14
Few stools							4				
Loose stool											7-12,14
Yellow/brown stained fur - perianal region											7-14
Red colored urine						2					
No abnormalities		0	0	0	0	0	0	0	0	0	0

TABLE 30

INDIVIDUAL NECROPSY FINDINGS

ACUTE DERMAL TOXICITY STUDY IN RABBITS

TEST ARTICLE: RED MIXTURE

Animal Number	Sex	Abnormalities Noted at Necropsy (Organ - Abnormality)
BB9789	M	Treated Skin - Discolored, pink, diffuse, mild
BB9800	M	Treated Skin - Fur, discoloration, diffuse, pink, mild
BB9801	M	Treated Skin - Fur near treated site tinged pink, diffuse, mild
BB9802	M	Treated Skin - Discoloration, diffuse, pink, moderate
BB9806	M	Treated Skin - Discolored, pink, diffuse, mild
BB9817	F	No abnormalities
BB9824	F	Treated Skin - Fur near treated site tinged pink, diffuse, mild
BB9831	F	Treated Skin - Discoloration, diffuse, pink, moderate
BB9833	F	Treated Skin - Fur near treated site tinged pink, diffuse, mild
BB9834	F	No abnormalities

TABLE 31

INDIVIDUAL BODY WEIGHT AND TEST ARTICLE ADMINISTRATION DATA

ACUTE ORAL TOXICITY STUDY IN RATS

TEST ARTICLE: DISPERSE RED 11 - LOT I

Animal Number	Sex	Body Weight (grams) Day of Study* 0	3	4	5	6	7	10	14	Amount of Test Article Administered (mg)	(ml)
AH0418	M	206	188	(185)	-	-	-	-	-	1,020	3.4
AH0419	M	199	179	(177)	-	-	-	-	-	990	3.3
AH0420	M	196	176	-	(170)	-	-	-	-	990	3.3
AH0421	M	197	172	(165)	-	-	-	-	-	990	3.3
AH0422	M	197	174	-	-	(153)	-	-	-	990	3.3
Mean		199	178							996	3.3
S.D.		4	6							13	0.0
S.E.		2	3							6	0.0
AH0461	F	143	141	-	-	-	153	160	163	720	2.4
AH0462	F	141	137	-	-	-	153	163	165	720	2.4
AH0463	F	139	135	-	-	-	150	159	162	690	2.3
AH0464	F	138	134	-	-	-	141	150	160	690	2.3
AH0466	F	143	142	-	-	-	150	163	171	720	2.4
Mean		141	138				151	159	164	780	2.4
S.D.		2	4				6	5	4	16	0.1
S.E.		1	2				3	2	2	7	0.0

Day 0 denotes fasted body weight the day of dose administration; day 14 denotes final body weight prior to final sacrifice; values in parentheses denote found dead body weights and are not included in the statistical analyses.

TABLE 32

INDIVIDUAL ANTEMORTEM OBSERVATIONS

ACUTE ORAL TOXICITY STUDY IN RATS

TEST ARTICLE: DISPERSE RED 11 - LOT 1

		Day(s) Finding Observed									
Finding	Animal No.: Sex:	AH0418 (M)	AH0419 (M)	AH0420 (M)	AH0421 (M)	AH0422 (M)	AH0461 (F)	AH0462 (F)	AH0463 (F)	AH0464 (F)	AH0466 (F)
Loose stools						8					
Purple colored urine		0-3	0-3	0-4	0-4	0-5	0-6	0-6	0-6	0-6	0-6
Purple colored perianal region						8	8-14	8-14	8-14	8,10-14	8-14
Skin discolored purple		0-3	0-3	0-4	0-4	0-5	0-9	0-9	0-9	0-9	0-9
Lethargy					4						
Poor coat quality						4,5					
Crusty eyes					4	5					
Crusty nose						5					
Purple colored tail									10,11		
Death		4	4	5	4	6					

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TABLE 33

INDIVIDUAL NECROPSY FINDINGS

ACUTE ORAL TOXICITY STUDY IN RATS

TEST ARTICLE: DISPERSE RED 11 - LOT 1

Animal Number	Sex	Abnormalities Noted at Necropsy (Organ - Abnormality)
AH0418*	M	Posterior Extremities - Pink in color Fat - Pink in color Liver - Green in color Stomach - Filled with dark fluid Intestines - Filled with dark fluid Testicular Fat - Pink in color Bladder - Distended, filled with pink fluid Musculature - Pink in color
AH0419*	M	Skin on Posterior Appendages - Blue-pink in color Liver - Mottled green Stomach - Filled with dark fluid Intestines - Filled with dark fluid Bladder - Distended, filled with pink fluid Musculature - Blue-pink in color
AH0420*	M	Skin - Pink in color Fat - Pink in color Liver - Discoloration, diffuse, dark grey; granular appearance Urinary Bladder - Contained pink fluid Intestine - Dark contents
AH0421*	M	Skin - Pink in color Fat - Pink in color Liver - Discoloration, dark grey, severe; granular appearance Urinary Bladder - Contained pink fluid Glandular Stomach - Discoloration, multiple focal, black, mucosa Intestine - Dark contents
AH0422*	M	Skin - Pale pink Intestine - Dark contents Pancreas - Pale Testes - Discoloration, diffuse, pink, bilateral Liver - Discoloration, diffuse, dark grey; granular appearance Subcutaneous Tissues - Hemorrhage, bilateral posterior appendage (mild), left anterior appendage (severe) and head (moderate) Fat - Discolored, pale pink

* Animal found dead

TABLE 33 (continued)

INDIVIDUAL NECROPSY FINDINGS

ACUTE ORAL TOXICITY STUDY IN RATS

TEST ARTICLE: DISPERSE RED 11 - LOT 1

Animal Number	Sex	Abnormalities Noted at Necropsy (Organ - Abnormality)
AH0461	F	External Surfaces - Purple stained fur, perineum
AH0462	F	External Surfaces - Purple stained fur, perineum
AH0463	F	External Surfaces - Purple stained fur, perineum
AH0464	F	External Surfaces - Purple stained fur, perineum
AH0466	F	External Surfaces - Purple stained fur, perineum

TABLE 34

INDIVIDUAL BODY WEIGHT AND TEST ARTICLE ADMINISTRATION DATA

ACUTE ORAL TOXICITY STUDY IN RATS

TEST ARTICLE: DISPERSE RED 11 - LOT 2

Animal Number	Sex	Body Weight (grams) Day of Study* 0	3	4	5	7	10	14	Amount of Test Article Administered (mg)	(ml)
AH0412	M	186	172	-	(154)	-	-	-	930	3.1
AH0413	M	189	178	(175)	-	-	-	-	960	3.2
AH0414	M	202	183	-	(172)	-	-	-	1,020	3.4
AH0415	M	198	182	(177)	-	-	-	-	990	3.3
AH0416	M	196	181	(178)	-	-	-	-	990	3.3
Mean		194	179						970	3.3
S.D.		7	4						34	0.1
S.E.		3	2						15	0.1
AH0452	F	138	124	-	-	138	150	156	690	2.3
AH0453	F	139	139	-	-	141	154	164	690	2.3
AH0455	F	141	143	-	-	153	159	165	720	2.4
AH0457	F	140	143	-	-	157	165	171	690	2.3
AH0459	F	138	130	-	-	112	132	151	690	2.3
Mean		139	136			140	152	161	696	2.3
S.D.		1	8			18	13	8	13	0.0
S.E.		1	4			8	6	4	6	0.0

* Day 0 denotes fasted body weight the day of dose administration; day 14 denotes final body weight prior to final sacrifice; values in parentheses denote found dead body weights and are not included in the statistical analyses.

TABLE 35

INDIVIDUAL ANTEMORTEM OBSERVATIONS

ACUTE ORAL TOXICITY STUDY IN RATS

TEST ARTICLE: DISPERSE RED 11 - LOT 2

Finding	Animal No.: Sex:	AH8412 (M)	AH8413 (M)	AH8414 (M)	AH8415 (M)	AH8416 (M)	AH8452 (F)	AH8453 (F)	AH8455 (F)	AH8457 (F)	AH8459 (F)
		Day(s) Finding Observed									
Red colored urine		0-4	0,2,3	0,2-4	0,2,3	0,2,3	0,2-4	0-5	0-4	0-4	0-4
Skin discolored pink		0-5	0-3	0-4	0-3	0-3	0-5	0-5	0-5	0-5	0-5
Dark colored stool		1-5	1-3	1-4	1-3	1-3	1,2	1,2	1,2	1,2	1,2
Lethargy		4									5,6
Ataxia		4									5,6
Prostration		5									
Irregular breathing		5									
Lacrimation											5
Crusty eye											6-13
Crusty muzzle											6
Few stools											6,7
Gasping											6
Red stained fur - perianal region		5						1-14	1-14		5-14
Red stained fur - ventral body											11
Red stain on tail								5-9	5-9		8,9
Death		5	4	5	4	4					
No abnormalities							6-14			6-14	

TABLE 36

INDIVIDUAL NECROPSY FINDINGS

ACUTE ORAL TOXICITY STUDY IN RATS

TEST ARTICLE: DISPERSE RED 11 - LOT 2

Animal Number	Sex	Abnormalities Noted at Necropsy (Organ - Abnormality)
AH0412*	M	Liver - Discoloration, diffuse, grey-green; granular appearance Glandular Stomach - Discoloration, multiple focal, red, mucosa Small Intestine - Dark contents Cecum - Dark contents Fat - Discoloration, pale pink Urinary Bladder - Contained pink fluid Appendages (right) - Severe subcutaneous hemorrhage Skin - Discoloration, diffuse, pale pink
AH0413*	M	Stomach - Filled with dark, thick material Intestines - Filled with dark fluid Abdominal Cavity - Filled with red fluid Urinary Bladder - Filled with pink fluid Extremities - Blue-pink in color External Surface - Perianal region covered with purple fluid
AH0414*	M	Liver - Discoloration, diffuse, dark grey; granular appearance Glandular Stomach - Discoloration, multiple focal, brown, mucosa Abdominal Fat - Pink discoloration Appendage (right posterior) - Severe subcutaneous hemorrhage Foot (left posterior) - Swollen; discoloration, diffuse, dark blue Skin - Pink External Surface - Red, crusty material around nose, mouth, and right ear.
AH0415*	M	Liver - Green Stomach - Filled with dark fluid Intestines - Filled with dark material Testicular Fat - Pink Urinary Bladder - Filled with pink fluid; distended Musculature - Pink in color Extremities - Blue in color

* Animal found dead

TABLE 36 (continued)

INDIVIDUAL NECROPSY FINDINGS

ACUTE ORAL TOXICITY STUDY IN RATS

TEST ARTICLE: DISPERSE RED 11 - LOT 2

Animal Number	Sex	Abnormalities Noted at Necropsy (Organ - Abnormality)
AH0416*	M	Liver - Green in color Stomach - Filled with dark, thick substance Intestines - Filled with dark, thick substance Lungs - Filled with dark substance Urinary Bladder - Distended, filled with purple fluid Abdominal Fat - Pink in color Testicular Fat - Pink External Surface - Red fluid around nose and mouth
AH0452	F	None
AH0453	F	Liver - Diaphragmatic hernia External Surface - Pink stained fur, perineum
AH0455	F	External Surface - Pink stained fur, perineum
AH0457	F	External Surface - Pink stained fur, perineum
AH0459	F	External Surface - Pink stained fur, perineum

* Animal found dead

TABLE 37

INDIVIDUAL BODY WEIGHT AND TEST ARTICLE ADMINISTRATION DATA

ACUTE ORAL TOXICITY STUDY IN RATS

TEST ARTICLE: DISPERSE BLUE 3

Animal Number	Sex	Body Weight (grams) Day of Study* 0	3	7	10	14	Amount of Test Article Administered (mg)	(ml)
AH0405	M	186	164	155	174	203	930	3.1
AH0406	M	196	175	168	191	220	990	3.3
AH0407	M	186	167	(137)	-	-	930	3.1
AH0408	M	198	186	189	213	232	990	3.3
AH0409	M	205	191	202	219	240	1,020	3.4
Mean		194	177	179	199	224	972	3.2
S.D.		8	12	21	21	16	48	0.1
S.E.		4	5	11	10	8	18	0.1
AH0434	F	141	(130)	-	-	-	690	2.3
AH0438	F	141	131	131	149	160	690	2.3
AH0440	F	141	133	127	140	153	690	2.3
AH0447	F	156	141	141	156	169	780	2.6
AH0449	F	154	139	138	147	161	780	2.6
Mean		147	136	134	148	161	726	2.4
S.D.		8	5	6	7	7	49	0.2
S.E.		3	2	3	3	3	22	0.1

* Day 0 denotes fasted body weight the day of dose administration; day 14 denotes final body weight prior to final sacrifice; values in parentheses denote found dead body weights and are not included in the statistical analyses.

TABLE 38

INDIVIDUAL ANTEMORTEM OBSERVATIONS

ACUTE ORAL TOXICITY STUDY IN RATS

TEST ARTICLE: DISPERSE BLUE 3

Finding	Animal No.: Sex:	AH0405 (M)	AH0406 (M)	AH0407 (M)	AH0408 (M)	AH0409 (M)	AH0434 (F)	AH0438 (F)	AH0440 (F)	AH0447 (F)	AH0449 (F)
		Day(s) Finding Observed									
Blue colored urine		0-7	0-7	0-6	0-7	0-7	0,2	0-7	0-8	0-7	0-7
Skin discolored blue		1-6	1-6	1-6	1-6	1-6	1,2	0-7	0-6	0-7	0-7
Crusty nose		3,4						3-5			3,4
Emaciation		5-8		6							
Poor coat quality		5-11	6-8	5,6							
Crusty Eyes				5,6							2-6
Sensitive to touch		10									
Blue stained tail						10-14					10-14
Lethargy							2				
Ataxia							2	2			2
Blue stained fur - perianal region										5-14	6-14
Death				7			3				
No abnormalities		12-14	9-14		8-14	8-9		8-14	9-14		

TABLE 39

INDIVIDUAL NECROPSY FINDINGS

ACUTE ORAL TOXICITY STUDY IN RATS

TEST ARTICLE: DISPERSE BLUE 3

Animal Number	Sex	Abnormalities Noted at Necropsy (Organ - Abnormality)
AH0405	M	Tail - Scattered purple areas Liver - Discolored, dark green Testicular Fat - Discolored, gray
AH0406	M	Tail - Scattered purple areas Liver - Discolored, dark green Testicular Fat - Discolored, grey
AH0407*	M	Liver - Discoloration, diffuse, grey green Intestine and Stomach - Dark contents Urinary Bladder - Contains purple fluid Lymph Node - Discoloration, red, cervical nodes Posterior Appendages - Subcutaneous hemorrhage, bilateral Fat - Discoloration, diffuse, grey, testicular fat
AH0408	M	Tail - Scattered purple areas Testicular Fat - Discolored, grey
AH0409	M	Tail - Scattered purple areas Liver - Discolored, dark green Testicular Fat - Discolored, grey
AH0434*	F	Stomach - Stained blue; dark contents Intestines - Dark contents Liver - Pale Musculature - Varying degrees of blue discoloration External Surfaces - Skin blue in color
AH0438	F	Tail - Scattered purple areas Liver - Discolored, brown
AH0440	F	Tail - Scattered purple areas External Surface - Purple stained fur - perineum
AH0447	F	Fat - Discolored, grey External Surface - Purple stained fur - perineum Tail - Scattered purple areas
AH0449	F	Fat - Discolored, grey External Surface - Purple stained fur - perineum Tail - Scattered purple areas

* Animal found dead

TABLE 48

INDIVIDUAL BODY WEIGHT AND TEST ARTICLE ADMINISTRATION DATA

ACUTE ORAL TOXICITY STUDY IN RATS

TEST ARTICLE: VIOLET MIXTURE

Animal Number	Sex	Body Weight (grams) Day of Study* 0	2	3	3	Amount of Test Article Administered (mg)	(ml)
AH1128	M	230	-	(222)		1,140	3.0
AH1129	M	227	(220)	-		1,140	3.0
AH1130	M	227	-	(215)		1,140	3.0
AH1131	M	235	-	221	(220)	1,170	3.9
AH1132	M	229	(220)	-		1,140	3.0
Mean		230				1,146	3.0
S.D.		3				13	0.0
S.E.		1				6	0.0
AH1154	F	160	-	(165)		840	2.0
AH1155	F	172	(173)	-		870	2.9
AH1156	F	170	(165)	-		840	2.0
AH1157	F	162	-	(150)		810	2.7
AH1158	F	170	(164)	-		840	2.0
Mean		168				840	2.0
S.D.		4				21	0.1
S.E.		2				9	0.0

* Day 0 denotes fasted body weight the day of dose administration; day 14 denotes final body weight prior to final sacrifice; values in parentheses denote found dead body weights and are not included in the statistical analyses.

TABLE 41

INDIVIDUAL ANTEMORTEM OBSERVATIONS

ACUTE ORAL TOXICITY STUDY IN RATS

TEST ARTICLE: VIOLET MIXTURE

Finding	Animal No.: / Sex:	AH1128 (M)	AH1129 (M)	AH1130 (M)	AH1131 (M)	AH1132 (M)	AH1154 (F)	AH1155 (F)	AH1156 (F)	AH1157 (F)	AH1158 (F)
		Day(s) Finding Observed									
Purple colored urine		0-2	0-2	0-2	0-3	0-2	0-2	0-2	0-2	0-2	0-2
Purple colored skin		0-2	0-2	0-2	0-3	0-2	0-2	0-2	0-2	0-2	0-2
Crusty eye					3			2	0-2		
Crusty nose		1,2			1,2	1,2	1,2	1,2	1,2		1
Lethargy					3						
Ataxia					3						
Purple colored fur - perianal region							1,2	1,2	1,2	1,2	1,2
Death		3	2	3	3	2	3	2	2	3	2

TABLE 42

INDIVIDUAL NECROPSY FINDINGS

ACUTE ORAL TOXICITY STUDY IN RATS

TEST ARTICLE: VIOLET MIXTURE

Animal Number	Sex	Abnormalities Noted at Necropsy (Organ - Abnormality)
AH1128*	M	All Mucocutaneous Junctions - Discolored purple Feet - Discolored purple Gastrointestinal Tract - Discolored purple; contents discolored purple Upper Respiratory Tract - Discolored purple All Mesenteric Surfaces - Discolored purple Cornea - Discolored purple Lung - Intensely red Liver - Dark brown
AH1129*	M	All Appendages - Purple in color Liver - Dark, mottled Stomach - Distended with dark purple contents Mesentery - Purple Intestines - Filled with purple material
AH1130*	M	All Mucocutaneous Junctions - Discolored purple Feet - Discolored purple Gastrointestinal Tract - Discolored purple; contents discolored purple Upper Respiratory Tract - Discolored purple All Mesenteric Surfaces - Discolored purple Cornea - Discolored purple Lung - Intensely red Liver - Dark brown
AH1131*	M	All Mucocutaneous Junctions - Discolored purple Feet - Discolored purple Gastrointestinal Tract - Discolored purple; contents discolored purple Upper Respiratory Tract - Discolored purple All Mesenteric Surfaces - Discolored purple Cornea - Discolored purple Lung - Intensely red Liver - Dark brown

* Animal found dead

TABLE 42 (continued)

INDIVIDUAL NECROPSY FINDINGS

ACUTE ORAL TOXICITY STUDY IN RATS

TEST ARTICLE: VIOLET MIXTURE

Animal Number	Sex	Abnormalities Noted at Necropsy (Organ - Abnormality)
AH1132*	M	All Appendages - Purple in color Lungs - Purple scattered areas; pale Liver - Grey mottled discoloration Stomach - Distended with dark contents Intestines - Filled with purple material Urinary Bladder - Filled with purple material Mesentery - Purple
AH1154*	F	All Mucocutaneous Junctions - Discolored purple Feet - Discolored purple Gastrointestinal Tract - Discolored purple; contents discolored purple Upper Respiratory Tract - Discolored purple All Mesenteric Surfaces - Discolored purple Cornea - Discolored purple Lung - Intensely red Liver - Dark brown
AH1155*	F	Posterior and Anterior Appendages - Purple Liver - Red, mottled appearance Stomach - Distended, filled with dark contents Intestines - Purple contents Mesentery - Purple
AH1156*	F	All Skin - Dark purple Liver - Prominent lobular pattern All Body Fat - Dark purple
AH1157*	F	All Mucocutaneous Junctions - Discolored purple Feet - Discolored purple Gastrointestinal Tract - Discolored purple; contents discolored purple Upper Respiratory Tract - Discolored purple All Mesenteric Surfaces - Discolored purple Cornea - Discolored purple Lung - Intensely red Liver - Dark brown
AH1158*	F	All Skin - Dark purple Liver - Prominent lobular pattern All Body Fat - Dark purple

* Animal found dead

TABLE 43

INDIVIDUAL BODY WEIGHT AND TEST ARTICLE ADMINISTRATION DATA

ACUTE ORAL TOXICITY STUDY IN RATS

TEST ARTICLE: SOLVENT RED 1

Animal Number	Sex	Body Weight (grams) Day of Study* 0	3	7	10	14	Amount of Test Article Administered (mg)	(ml)
AH2106	M	213	235	237	248	252	1,062	5.9
AH2113	M	208	228	231	244	248	1,044	5.8
AH2114	M	216	231	236	247	250	1,080	6.0
AH2115	M	215	236	240	250	257	1,080	6.0
AH2116	M	221	240	246	263	267	1,098	6.1
Mean		215	234	238	250	255	1,073	6.0
S.D.		5	5	6	7	8	21	0.1
S.E.		2	2	2	3	3	9	0.1
AH1170	F	179	186	189	199	197	980	5.0
AH1173	F	178	190	188	196	196	882	4.9
AH1174	F	174	185	183	189	186	864	4.8
AH1175	F	179	187	187	193	193	900	5.0
AH1176	F	183	197	194	198	198	918	5.1
Mean		179	189	188	195	194	893	5.0
S.D.		3	5	4	4	5	21	0.1
S.E.		1	2	2	2	2	9	0.1

* Day 0 denotes fasted body weight the day of dose administration; day 14 denotes final body weight prior to final sacrifice; values in parentheses denote found dead body weights and are not included in the statistical analyses.

TABLE 44

INDIVIDUAL ANTEMORTEM OBSERVATIONS

ACUTE ORAL TOXICITY STUDY IN RATS

TEST ARTICLE: SOLVENT RED 1

Finding	Animal No.: Sex:	AH2106 (M)	AH2113 (M)	AH2114 (M)	AH2115 (M)	AH2116 (M)	AH1170 (F)	AH1173 (F)	AH1174 (F)	AH1175 (F)	AH1176 (F)
					Day(s) Finding Observed						
Red colored urine		0,1	0,1	0-2	0,1	0,1	0,1	0,1	0,1	0,1	0,1
Red colored stool		0-2	1,2	0-2	1,2	0-2	1,2	1,2	0-2	0-2	1,2
Loose stool		0		0		0					
Red stained fur -											
perianal region		0,2-13	0-2	0,3-14	1-13	0,6-14	1-14	1-14	3-14	1-14	1-14
head		1		1,2		1-5			1,2		
ventral portion of body		1		1,2		1-5			1,2		
muzzle			0								
Red colored stain on tail		1-14		1-14	1-13	1-14			1-14	1	1-14
No abnormalities			3-14		14						

TABLE 45

INDIVIDUAL NECROPSY FINDINGS

ACUTE ORAL TOXICITY STUDY IN RATS

TEST ARTICLE: SOLVENT RED 1

Animal Number	Sex	Abnormalities Noted at Necropsy (Organ - Abnormality)
AH2106	M	None
AH2113	M	None
AH2114	M	None
AH2115	M	None
AH2116	M	None
AH1170	F	None
AH1173	F	None
AH1174	F	None
AH1175	F	None
AH1176	F	None

TABLE 46

INDIVIDUAL BODY WEIGHT AND TEST ARTICLE ADMINISTRATION DATA

ACUTE ORAL TOXICITY STUDY IN RATS

TEST ARTICLE: RED MIXTURE

Animal Number	Sex	Body Weight (grams) Day of Study* 0	3	4	7	10	14	Amount of Test Article Administered (mg)	(ml)
AH1135	M	216	226	-	226	237	241	1,080	5.4
AH1138	M	236	244	-	244	260	266	1,180	5.9
AH1139	M	228	215	(211)	-	-	-	1,140	5.7
AH1140	M	222	230	-	237	250	252	1,120	5.6
AH1141	M	234	248	-	246	255	258	1,180	5.9
Mean		227	233		238	251	254	1,140	5.7
S.D.		8	13		9	10	11	42	0.2
S.E.		4	6		5	5	5	19	0.1
AH1160	F	166	175	-	176	181	184	840	4.2
AH1161	F	167	176	-	173	182	185	840	4.2
AH1162	F	178	188	-	187	199	199	900	4.5
AH1163	F	175	188	-	188	193	193	880	4.4
AH1164	F	172	182	-	187	191	187	860	4.3
Mean		172	182		182	189	190	864	4.3
S.D.		5	6		7	8	6	26	0.1
S.E.		2	3		3	3	3	12	0.1

* Day 0 denotes fasted body weight the day of dose administration; day 14 denotes final body weight prior to final sacrifice; values in parentheses denote found dead body weights and are not included in the statistical analyses.

TABLE 47

INDIVIDUAL ANTEMORTEM OBSERVATIONS

ACUTE ORAL TOXICITY STUDY IN RATS

TEST ARTICLE: RED MIXTURE

Finding	Animal No.: Sex:	AH1135 (M)	AH1138 (M)	AH1139 (M)	AH1140 (M)	AH1141 (M)	AH1160 (F)	AH1161 (F)	AH1162 (F)	AH1163 (F)	AH1164 (F)
		Day(s) Finding Observed									
Red-violet colored urine		0-3	0-3	0-3	0-3	0-3	0-3	0-3	0-3	0-3	0-3
Red colored stool		1	0,1	0,1	0,1	1	1	1	0-2	1,2	1,2
Loose stool			0,1	0	0				0		
Skin discolored pink		1	1	1	1	1	1	1	1		1
Red stain on tail		1-10	1-14	1-3	1-14	1-14	1		1		
Red stain on feet		1,2	1-4	2,3	1,2	1					
Red stain on muzzle			1		1	1					
Red stained fur - perianal region		1-14	2-14	0,2,3	0-14	1-14	1-14	1-14	0-14	1-14	2-14
ventral surface of body			1	1,2							
dorsal surface of body				1,2							
Red-violet stained fur - perineum											1
Lethargy				3							
Scabby tail				3							
Crusty nose				3							
Crusty eye											2
Poor coat quality				3							
Death				4							

TABLE 48

INDIVIDUAL NECROPSY FINDINGS

ACUTE ORAL TOXICITY STUDY IN RATS

TEST ARTICLE: RED MIXTURE

Animal Number	Sex	Abnormalities Noted at Necropsy (Organ - Abnormality)
AH1135	M	External Surface - Purple staining of perineum
AH1138	M	Lung - Discoloration, multiple focal, dark red, right diaphragmatic lobe External Surface - Purple staining of perineum
AH1139*	M	Lung - Pale Gastrointestinal Tract - Contents discolored red Fat - Discolored red Mesentery - Discolored red Urinary Bladder - Discolored contents, red External Surface - Crusting on feet and tail
AH1140	M	External Surface - Purple staining of perineum
AH1141	M	Lung - Discoloration, solitary, dark red, right diaphragmatic lobe External Surface - Purple staining of perineum
AH1160	F	External Surface - Purple staining of perineum
AH1161	F	External Surface - Purple staining of perineum
AH1162	F	External Surface - Purple staining of perineum
AH1163	F	External Surface - Purple staining of perineum
AH1164	F	External Surface - Purple staining of perineum

* Animal found dead

TABLE 49

INDIVIDUAL BODY WEIGHT AND TEST ARTICLE ADMINISTRATION DATA

ACUTE ORAL LD_{50} STUDY IN RATS

TEST ARTICLE: DISPERSE RED 11 - LOT 1

DOSE GROUP: 562 mg/kg

Animal Number	Sex	Body Weight (grams) Day of Study* 0	3	7	10	14	Amount of Test Article Administered (mg)	(ml)
AH2132	M	219	210	228	238	245	122	3.7
AH2133	M	217	211	224	234	244	122	3.7
AH2135	M	236	238	253	266	275	132	4.0
AH2136	M	218	212	226	235	248	122	3.7
AH2137	M	232	229	246	253	256	129	3.9
Mean		224	220	235	245	252	125	3.8
S.D.		9	13	13	14	14	5	0.1
S.E.		4	6	6	6	6	2	0.1

* Day 0 denotes fasted body weight the day of dose administration; day 14 denotes final body weight prior to final sacrifice

TABLE 49 (continued)

INDIVIDUAL BODY WEIGHT AND TEST ARTICLE ADMINISTRATION DATA

ACUTE ORAL LD_{50} STUDY IN RATS

TEST ARTICLE: DISPERSE RED 11 - LOT 1

DOSE GROUP: 700 mg/kg

Animal Number	Sex	Body Weight (grams) Day of Study* 0	3	7	10	14	Amount of Test Article Administered (mg)	(ml)
AH3427	M	217	203	215	229	240	154	3.7
AH3428	M	225	222	234	247	262	158	3.8
AH3429	M	217	200	222	241	252	154	3.7
AH3430	M	224	211	210	226	247	158	3.8
AH3431	M	218	201	198	217	236	154	3.7
Mean		220	207	216	232	247	156	3.7
S.D.		4	9	13	12	10	2	0.1
S.E.		2	4	6	5	5	1	0.0

* Day 0 denotes fasted body weight the day of dose administration; day 14 denotes final body weight prior to final sacrifice;

TABLE 49 (CONTINUED)

INDIVIDUAL BODY WEIGHT AND TEST ARTICLE ADMINISTRATION DATA

ACUTE ORAL LD_{50} STUDY IN RATS

TEST ARTICLE: DISPERSE RED 11 - LOT 1

DOSE GROUP: 891 mg/kg

Animal Number	Sex	Body Weight (grams) Day of Study* 0	3	5	6	7	10	14	Amount of Test Article Administered (mg)	(ml)
AH2101	M	192	179	-	(158)	-	-	-	173	3.3
AH2102	M	194	179	-	(152)	-	-	-	173	3.3
AH2103	M	196	179	-	(156)	-	-	-	173	3.3
AH2104	M	193	179	(162)	-	-	-	-	173	3.3
AH2105	M	207	193	-	-	204	230	251	183	3.5
Mean		196	182						175	3.3
S.D.		6	6						4	0.1
S.E.		3	3						2	0.0

* Day 0 denotes fasted body weight the day of dose administration; day 14 denotes final body weight prior to final sacrifice; values in parentheses denote found dead body weights and are not included in the statistical analyses.
- = Not applicable

TABLE 49 (continued)

INDIVIDUAL BODY WEIGHT AND TEST ARTICLE ADMINISTRATION DATA

ACUTE ORAL LD_{50} STUDY IN RATS

TEST ARTICLE: DISPERSE RED 11 - LOT 1

DOSE GROUP: 1,413 mg/kg

Animal Number	Sex	Body Weight (grams) Day of Study* 0	3	4	5	6	Amount of Test Article Administered (mg)	(ml)
AH2092	M	176	169	(167)	-	-	249	3.0
AH2093	M	187	170	-	-	(155)	266	3.2
AH2094	M	175	160	-	(136)	-	249	3.0
AH2095	M	179	163	(157)	-	-	249	3.0
AH2096	M	186	171	-	(157)	-	266	3.2
Mean		181	167				256	3.1
S.D.		6	5				9	0.1
S.E.		3	2				4	0.0

* Day 0 denotes fasted body weight the day of dose administration; day 14 denotes final body weight prior to final sacrifice; values in parentheses denote found dead body weights and are not included in the statistical analyses.
- = Not applicable

TABLE 50

INDIVIDUAL ANTEMORTEM OBSERVATIONS

ACUTE ORAL LD_{50} STUDY IN RATS

TEST ARTICLE: DISPERSE RED 11 - LOT 1

DOSE GROUP: 562 mg/kg

Finding	Animal No.: Sex:	Day(s) Finding Observed AH2132 (M)	AH2133 (M)	AH2135 (M)	AH2136 (M)	AH2137 (M)
Purple colored urine		0-4	0-4	0-4	0-4	0-4
Skin discolored purple		0-2	0-2	0-2	0-2	0-2
Purple stained fur - perianal region		0-14	1-14	0-14	0-14	0-14
Purple loose stools		0		0	0	0

TABLE 5B (continued)

INDIVIDUAL ANTEMORTEM OBSERVATIONS

ACUTE ORAL LD_{50} STUDY IN RATS

TEST ARTICLE: DISPERSE RED 11 - LOT 1

DOSE GROUP: 700 mg/kg

Finding	Animal No.: / Sex:	AH3427 (M)	AH3428 (M)	Day(s) Finding Observed AH3429 (M)	AH3430 (M)	AH3431 (M)
Purple colored urine		0-5	0-5	0-5	0-5	0-5
Loose stools		0				
Purple stained fur - perianal region		0-14	0-14	1-7	1,2,4-7	1-14
Skin discolored purple		1,2	1,2	1,2	1,2	1,2
Crusty eye			4		4,5	4-8
Lethargy			4,5			
Red crusty substance around ear tag						4,5, 8-10
Crusty substance around ear tag						6,7
No abnormalities				8-14	8-14	

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TABLE 50 (continued)

INDIVIDUAL ANTEMORTEM OBSERVATIONS

ACUTE ORAL LD_{50} STUDY IN RATS

TEST ARTICLE: DISPERSE RED 11 - LOT 1

DOSE GROUP: 891 mg/kg

Finding	Animal No.: / Sex:	Day(s) Finding Observed				
		AH2101 (M)	AH2102 (M)	AH2103 (M)	A32104 (M)	AH2105 (M)
Purple colored urine		0-5	0-5	0-5	0-4	0-5
Skin discolored purple		0,1	0,1	0-4	0,1	0,1
Purple stained fur - perianal region			0-5	2-5	2-4	2-14
Loose stools			0			
Crusty substance around ear tag		2		3-5		
Red crusty substance around ear tag		3-5	3-5		3,4	
Crusty nose		3-5	5			
Lethargy		4,5	4,5	5	4	
Ataxia		4,5	5		4	
Squinting		4			4	
Pale		4,5	4,5	5	4	
Irregular breathing		5				
Crusty eye				5		5
Death		6	6	6	5	

TABLE 5@ (continued)

INDIVIDUAL ANTEMORTEM OBSERVATIONS

ACUTE ORAL $LD_{5@}$ STUDY IN RATS

TEST ARTICLE: DISPERSE RED 11 - LOT 1

DOSE GROUP: 1,413 mg/kg

Finding	Animal No.: Sex:	Day(s) Finding Observed AH2@92 (M)	AH2@93 (M)	AH2@94 (M)	AH2@95 (M)	AH2@96 (M)
Purple colored urine		@,1	@-5	@,1,4-5	@,1,3,4	@,1,3,4
Skin discolored purple		@-3	@-5	@-5	@-1	@-4
Purple colored loose stools					@	
Purple stained fur - perianal region		1-3	1-5	1-5	@-4	1-4
Lethargy		3	5	5		
No stools		3				
Red stained fur - all feet			5			
Red crusty substance around ear tag				3-5		
Pale				5	4	
Slow respirations					4	
Prostration					4	
Sensitive to touch						3
Crusty eye						4
Death		4	6	5	4	5

TABLE 51

INDIVIDUAL NECROPSY FINDINGS

ACUTE ORAL LD_{50} STUDY IN RATS

TEST ARTICLE: DISPERSE RED 11 - LOT 1

DOSE GROUP: 562 mg/kg

Animal Number	Sex	Abnormalities Noted at Necropsy (Organ - Abnormality)
AH2132	M	External Surface - Purple stained fur, perianal region Liver - Discoloration, diffuse, grey-green
AH2133	M	External Surface - Purple stained fur, perianal region Liver - Discoloration, diffuse, grey-green
AH2135	M	External Surface - Purple stained fur, perianal region
AH2136	M	External Surface - Purple stained fur, perianal region Liver - Discoloration, diffuse, grey-green
AH2137	M	External Surface - Purple stained fur, perianal region Liver - Discoloration, diffuse, grey-green

TABLE 51 (continued)

INDIVIDUAL NECROPSY FINDINGS

ACUTE ORAL LD_{50} STUDY IN RATS

TEST ARTICLE: DISPERSE RED 11 -- LOT 1

DOSE GROUP: 708 mg/kg

Animal Number	Sex	Abnormalities Noted at Necropsy (Organ - Abnormality)
AH3427	M	External Surface - Purple stained fur - perianal region Liver - Discoloration, diffuse, dark brown
AH3428	M	External Surface - Discoloration, diffuse, perianal region Liver - Discoloration, diffuse, dark brown
AH3429	M	Liver - Discoloration, diffuse, dark brown
AH3430	M	Liver - Discoloration, diffuse, brown grey
AH3431	M	External Surface - Purple stained fur, perianal region Liver - Discoloration, diffuse, brown grey

*Animal found dead prior to final sacrifice.

TABLE 51 (continued)

INDIVIDUAL NECROPSY FINDINGS

ACUTE ORAL LD_{50} STUDY IN RATS

TEST ARTICLE: DISPERSE RED 11 - LOT 1

DOSE GROUP: 891 mg/kg

Animal Number	Sex	Abnormalities Noted at Necropsy (Organ - Abnormality)
AH2101*	M	External Surface - Pink discoloration, perianal region Liver - Discoloration, diffuse, dark grey Spleen - Discoloration, diffuse, pale Testes - Discoloration, diffuse, red Fat - Discoloration, diffuse, pink Left Posterior Appendage - Subcutaneous hemorrhage
AH2102*	M	External Surface - Pink discoloration, perianal region Liver - Discoloration, diffuse, grey Spleen - Discoloration, diffuse, pale Testis - Discoloration, diffuse, red, left Epididymides - Discoloration, diffuse, red Fat - Pale pink discoloration
AH2103*	M	External Surface - Pink discoloration, perianal region Liver - Discoloration, diffuse, grey Spleen - Discoloration, diffuse, pale Testes - Discoloration, diffuse, red Epididymides - Discoloration, diffuse, red Fat - Discoloration, diffuse, pink
AH2104*	M	External Surface - Light purple staining of fur in perineum Lung - 2 focal hemorrhages, right apical lobe Intestine - Contents purple Liver - Dark green with prominent lobular pattern Urinary Bladder - Contents pink Rear Leg Musculature - Bilateral hemorrhages Subcutaneous - Multiple hemorrhages Ear - Clotted blood around ear tag
AH2105	M	External Surface - Purple stained fur, perineum region

* Animal found dead prior to final sacrifice.

TABLE 51 (continued)

INDIVIDUAL NECROPSY FINDINGS

ACUTE ORAL LD_{50} STUDY IN RATS

TEST ARTICLE: DISPERSE RED 11 - LOT 1

DOSE GROUP: 1,413 mg/kg

Animal Number	Sex	Abnormalities Noted at Necropsy (Organ - Abnormality)
AH2092*	M	Anus - Discolored pink-red Lung - Pale Stomach - Contains copious red material Liver - Green
AH2093*	M	External Surface - Red brown crusted material around nose and mouth, on feet, and in perianal region Intestine - Dark contents Liver - Discoloration, diffuse, dark grey Testis - Discoloration, diffuse, pale red, bilateral Fat - Discoloration, diffuse, pink Non-Glandular Stomach - Discoloration, diffuse, red, serosa
AH2094*	M	External Surface - Purple discoloration, perianal region Cecum - Dark contents Colon - Dark contents Tissue Along Descending Aorta - Severe hemorrhage Testis - Discoloration, dark red, left Liver - Discoloration, diffuse, dark grey
AH2095*	M	External Surface - Discoloration, diffuse, pink, perianal region, and base of tail Left Posterior Appendage - Subcutaneous hemorrhage Liver - Discoloration, diffuse, dark grey Intestine - Dark contents Testis - Discoloration, diffuse, red, right Urinary Bladder - Distended with red fluid; discoloration, diffuse, red Stomach - Dark contents Glandular Stomach - Discoloration, multiple focal, brown, on mucosa Fat - Discoloration, diffuse, pink

* Animal found dead prior to final sacrifice

TABLE 51 (continued)

INDIVIDUAL NECROPSY FINDINGS

ACUTE ORAL LD_{50} STUDY IN RATS

TEST ARTICLE: DISPERSE RED 11 - LOT 1

DOSE GROUP: 1,413 mg/kg (cont.)

Animal Number	Sex	Abnormalities Noted at Necropsy (Organ - Abnormality)
AH2096*	M	Lung - Discoloration, solitary, red, left lobe Along Abdominal Aorta - Severe hemorrhage Liver - Discoloration, diffuse, dark grey Urinary Bladder - Distended with purple fluid Small Intestine - Discoloration, diffuse, purple Stomach - Dark contents Testicular Fat - Discoloration, diffuse, purple Right Posterior Appendage - Subcutaneous hemorrhage

* Animal found dead prior to final sacrifice.

TABLE 52

LITCHFIELD-WILCOXON LD_{50} FOR MALES

ACUTE ORAL LD_{50} STUDY IN RATS

TEST ARTICLE: DISPERSE RED 11 - LOT .

Dose (mg/kg)	Observed Deaths Proportion	Observed Deaths Percent	Expected Deaths Percent	Difference
562.0	0/5	0.0		
708.0	0/5	0.0		
891.0	4/5	80.0		
1,413.0	5/5	100.0		

Total number of animals: 20

At least two dose levels with percent observed death between 0 and 100 are required to calculate a least-squares regression. The LD-50 cannot be computed.

TABLE 53

INDIVIDUAL BODY WEIGHT AND TEST ARTICLE ADMINISTRATION DATA

ACUTE ORAL LD_{50} STUDY IN RATS

TEST ARTICLE: DISPERSE RED 11 - LOT 2

DOSE GROUP: 562 mg/kg

Animal Number	Sex	Body Weight (grams) Day of Study* 0	3	5	6	7	10	14	Amount of Test Article Administered (mg)	(ml)
AH2112	M	209	193	-	-	196	209	221	119	3.6
AH2125	M	218	200	-	-	203	220	239	122	3.7
AH2127	M	216	194	-	-	202	215	232	122	3.7
AH2139	M	229	217	-	-	236	250	261	129	3.9
AH2142	M	227	203	-	-	208	229	240	129	3.9
Mean		220	201			209	225	239	124	3.8
S.D.		8	10			16	16	15	5	0.1
S.E.		4	4			7	7	7	2	0.1

* Day 0 denotes fasted body weight the day of dose administration; day 14 denotes final body weight prior to final sacrifice; values in parentheses denote found dead body weights and are not included in the statistical analyses.

TABLE 53 (continued)

INDIVIDUAL BODY WEIGHT AND TEST ARTICLE ADMINISTRATION DATA

ACUTE ORAL LD_{50} STUDY IN RATS

TEST ARTICLE: DISPERSE RED II - LOT 2

DOSE GROUP: 691 mg/kg

Animal Number	Sex	Body Weight (grams) Day of Study* 0	3	5	6	7	10	14	Amount of Test Article Administered (mg)	(ml)
AH2117	M	231	211	-	(179)	-	-	-	208	4.0
AH2126	M	234	214	-	-	205	227	257	240	4.0
AH2129	M	230	215	(200)	-	-	-	-	213	4.1
AH2130	M	214	195	(173)	-	-	-	-	192	3.7
AH2131	M	233	218	-	-	221	239	259	208	4.0
Mean		230	211			215	233	258	206	4.0
S.D.		3	9			8	8	1	8	0.2
S.E.		4	4			6	6	1	4	0.1

* Day 0 denotes fasted body weight the day of dose administration; day 14 denotes final body weight prior to final sacrifice; values in parentheses denote found dead body weights and are not included in the statistical analyses.

TABLE 53 (continued)

INDIVIDUAL BODY WEIGHT AND TEST ARTICLE ADMINISTRATION DATA

ACUTE ORAL LD_{50} STUDY IN RATS

TEST ARTICLE: DISPERSE RED 11 - LOT 2

DOSE GROUP: 1,413 mg/kg

Animal Number	Sex	Body Weight (grams) Day of Study* 0	3	6	7	10	14	Amount of Test Article Administered (mg)	(ml)
AH2118	M	214	196	(173)	-	-	-	299	3.6
AH2119	M	220	204	-	195	215	237	307	3.7
AH2120	M	209	(194)	-	-	-	-	299	3.6
AH2124	M	203	193	-	196	211	222	291	3.5
AH2126	M	213	193	(165)	-	-	-	299	3.6
Mean		212	197		196	213	230	299	3.6
S.D.		6	5		1	3	11	6	0.1
S.E.		3	3		1	2	8	3	0.0

* Day 0 denotes fasted body weight the day of dose administration; day 14 denotes final body weight prior to final sacrifice; values in parentheses denote found dead body weights and are not included in the statistical analyses.

TABLE 54

INDIVIDUAL ANTEMORTEM OBSERVATIONS

ACUTE ORAL LD_{50} STUDY IN RATS

TEST ARTICLE: DISPERSE RED 11 - LOT 2

DOSE GROUP: 562 mg/kg

	Day(s) Finding Observed				
Finding / Animal No.: / Sex:	AH2112 (M)	AH2125 (M)	AH2127 (M)	AH2139 (M)	AH2142 (M)
Red-violet colored urine	0-5	0-5	0-3	0-3	0-3
Skin discolored pink	1-5	0-5	0-5	1,2	1-5
Loose stool	1		0		0
Yellow/brown stained fur - perianal region			0		0
Red-violet stained fur - perianal region	1-14	1-14	1-14	1-14	1-14
Red-violet stain on tail		6-14	6-9	3-14	
Crusty eye			4,5	3-6	4-6
Crusty nose	4,5		4		4
Poor coat quality		5			

TABLE 54 (continued)

INDIVIDUAL ANTEMORTEM OBSERVATIONS

ACUTE ORAL LD_{50} STUDY IN RATS

TEST ARTICLE: DISPERSE RED 11 - LOT 2

DOSE GROUP: 891 mg/kg

Finding / Animal No.: / Sex:	AH2117 (M)	AH2128 (M)	AH2129 (M)	AH2130 (M)	AH2131 (M)
	Day(s) Finding Observed				
Skin discolored pink	0-2	0-5	0-4	0-4	0-5
Red-violet colored urine	0-5	0-5	0-4	0-4	0-5
Red-violet stained muzzle				0	
Red-violet fur - perianal region	2-5	1-14	1-4	1-4	1-14
Red-violet stain on tail	2-5	2-14		3,4	2-14
Few stools	3,5	3-5	2-4	4	3
No stool	4			3	
Loose stool		1			
Crusty muzzle	2-5				
Crusty eye	5	4-7		4	4-9
Crusty nose				4	
Crusty substance around ear tag				4	
Pale	5			4	
Ataxia	4,5				
Left posterior foot scabby	3-5				
Death	6		5	5	

TABLE 54 (continued)

INDIVIDUAL ANTEMORTEM OBSERVATIONS

ACUTE ORAL LD_{50} STUDY IN RATS

TEST ARTICLE: DISPERSE RED 11 - LOT 2

DOSE GROUP: 1,413 mg/kg

Finding	Animal No.: Sex:	AH2118 (M)	AH2119 (M)	AH2120 (M)	AH2124 (M)	AH2126 (M)
		Day(s) Finding Observed				
Red-violet colored urine		0-5	0-5	0,1	0-5	0-5
Red-violet colored stool		0	0	0		
Loose stool		0	0	0		
Red-violet stained fur - perianal region		0-5	0-14	0,1	1-14	1-6
Skin discolored pink		0-4	0-4	0-2	0-4	0-4
Red-violet stain on tail		1-5	1-14	1		
Red stain on tail		5		2		5,6
Few stools		1	1,4	1	4	1-5
No stool		4,5				6
Pale		5				5,6
Lethargy		4,5		2		5,6
Poor coat quality			3-5			
Squinting		3-5	3	2	3,4	6
Crusty eye		4,5			3-9	4-6
Crusty muzzle		5		1,2		2-6
Red discharge from penis				2		
Red discharge on left posterior foot						2
Scab on left posterior foot						3-6
Death		6		3		6

TABLE 55

INDIVIDUAL NECROPSY FINDINGS

ACUTE ORAL LD_{50} STUDY IN RATS

TEST ARTICLE: DISPERSE RED 11 - LOT 2

DOSE GROUP: 562 mg/kg

Animal Number	Sex	Abnormalities Noted at Necropsy (Organ - Abnormality)
AH2112	M	Liver - Discoloration, diffuse, grey External Surface - Discoloration, diffuse, pink, pelvic region, on fur
AH2125	M	Liver - Discoloration, diffuse, grey External Surface - Discoloration, diffuse, pink, pelvic region, on fur
AH2127	M	Liver - Discoloration, diffuse, dark grey External Surface - Discoloration, diffuse, pink, pelvic region, on fur
AH2139	M	Liver - Discoloration, diffuse, grey External Surface - Discoloration, diffuse, pink, pelvic region and tail, on fur
AH2142	M	Liver - Discoloration, diffuse, grey External Surface - Discoloration, diffuse, pink, pelvic region, on fur

TABLE 55 (continued)

INDIVIDUAL NECROPSY FINDINGS

ACUTE ORAL LD_{50} STUDY IN RATS

TEST ARTICLE: DISPERSE RED 11 - LOT 2

DOSE GROUP: 891 mg/kg

Animal Number	Sex	Abnormalities Noted at Necropsy (Organ - Abnormality)
AH2117*	M	Liver - Discoloration, diffuse, green Stomach - Contents dark Testicular Fat - Discoloration, diffuse, pink Abdominal Fat - Discoloration, diffuse, pink Appendages (right side) - Discoloration, diffuse, red, subcutaneous External Surface - Discoloration, diffuse, black, pelvic region, crusted
AH2128	M	Liver - Discoloration, diffuse, dark green Skin - Scab, crusted, anterior to penis External Surface - Purple stained fur, perianal region
AH2129*	M	Liver - Discoloration, diffuse, green Abdominal Cavity and Viscera - Discoloration, diffuse, purple pink Pelvic Cavity and Viscera - Discoloration, diffuse, purple pink External Surface - Discoloration, diffuse, purple pink, pelvic region
AH2130*	M	Liver - Discoloration, diffuse, green Abdominal Cavity and Viscera - Discoloration, diffuse, purple pink Pelvic Cavity and Viscera - Discoloration, diffuse, purple pink External Surface - Discoloration, diffuse, purple pink, pelvic region
AH2131	M	Liver - Discoloration, diffuse, dark green External Surface - Purple stained fur, perianal region

* Animal found dead

TABLE 55 (continued)

INDIVIDUAL NECROPSY FINDINGS

ACUTE ORAL LD_{50} STUDY IN RATS

TEST ARTICLE: DISPERSE RED 11 - LOT 2

DOSE GROUP: 1,413 mg/kg

Animal Number	Sex	Abnormalities Noted at Necropsy (Organ - Abnormality)
AH2118*	M	Liver - Discoloration, diffuse, grey Stomach - Dark contents Cecum - Dark contents Colon - Dark contents Testes - Discoloration, diffuse, red, bilateral Urinary bladder - Distended with pink fluid Appendage (left anterior) - Subcutaneous hemorrhage External Surface - Pink stained fur, perianal region External Surface - Red crusty material, around nose and mouth
AH2119	M	Liver - Discoloration, diffuse, green grey External Surface - Discoloration, diffuse, purple pink, pelvic region, on fur
AH2120*	M	Liver - Dark in color Stomach - Contents dark Intestines - Contents dark Urinary Bladder - Contained dark purple fluid Appendages (posterior) - Mild subcutaneous hemorrhage Appendages - Pink in color Fat - Pink in color Tail - Pink in color External Surfaces - Red brown crusted material, perianal region and around nose and mouth
AH2124	M	Liver - Discoloration, diffuse, green grey
AH2126*	M	Liver - Green, severe Stomach - Abnormal contents, purple Intestine - Abnormal contents, purple Lung - Pale, severe Kidneys - Cortex pale Testes - Subcapsular hemorrhage Skeletal Muscle - Pale, marked Extremities (left and right) - Hematomas

* Animal found dead

TABLE 56

LITCHFIELD-WILCOXON LD_{50} FOR MALES

ACUTE ORAL LD_{50} STUDY IN RATS

TEST ARTICLE: DISPERSE RED 11 - LOT 2

Dose (mg/kg)	Observed Deaths Proportion	Observed Deaths Percent	Expected Deaths Percent	Difference
562.0	0/5	0.0(9.7)	14.9	-5.2
891.0	3/5	60.0	39.6	20.4
1,413.0	3/5	60.0	69.6	-9.6

Total number of animals: 15

NOTE - The values in parentheses are those used by the Litchfield-Wilcoxon method to compute Chi-Square contributions.

Calculated Chi-Square: 1.196

Critical Chi-Square (P = .05) for 1 degree of freedom: 3.956

The data are not significantly heterogeneous.

Calculated LD-50: 1042.7 mg/kg

95% Confidence Limits: 619.7 - 1754.3 mg/kg

The confidence limits are within 68.2% of the LD-50.

Slope: 3.88 (probits/log dose)

There are 10 animals included in groups with expected deaths between 16% (LD-16 = 575.7 mg/kg) and 84% (LD-84 = 1888.4 mg/kg).

Given the slope calculated from the present data, a total of 82 animals would be needed in groups with expected deaths between 16% and 84% in order to get the confidence limits within 20% of the LD-50. However, adding more test groups may change the value of the slope.

FIGURE E-1: DOSE-RESPONSE CURVE FOR MALES

ACUTE ORAL LD_{50} STUDY IN RATS

TEST ARTICLE: DISPERSE RED 11 - LOT 2

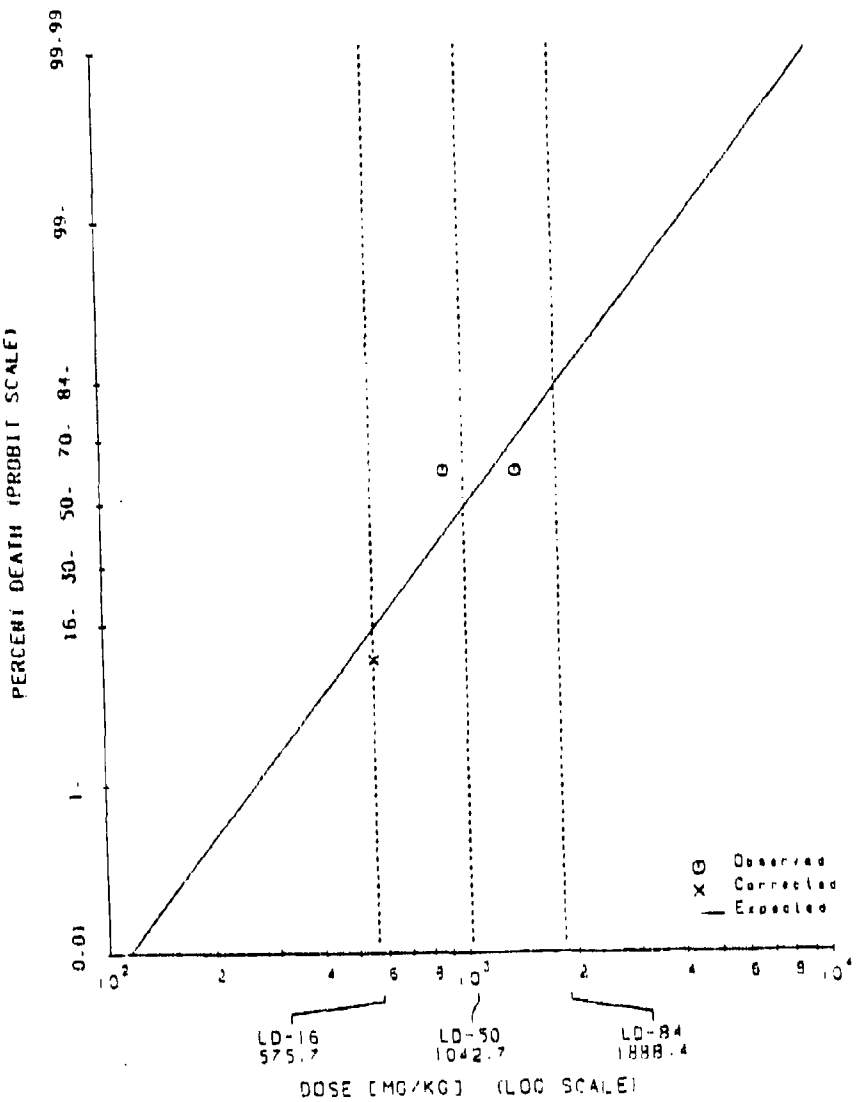

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TABLE 57

INDIVIDUAL BODY WEIGHT AND TEST ARTICLE ADMINISTRATION DATA

ACUTE ORAL LD_{50} STUDY IN RATS

TEST ARTICLE: VIOLET MIXTURE

DOSE GROUP: 794 mg/kg

Animal Number	Sex	Body Weight (grams) Day of Study* 0	3	5	7	10	14	Amount of Test Article Administered (mg)	(ml)
AH3435	M	231	210	-	201	226	253	182	3.9
AH3436	M	221	207	(196)	-	-	-	177	3.8
AH3437	M	237	224	-	221	243	263	187	4.0
AH3438	M	230	212	-	205	229	251	182	3.9
AH3443	M	227	214	-	198	218	243	182	3.9
Mean		229	213		206	229	253	182	3.9
S.D.		6	6		10	10	8	4	0.1
S.E.		3	3		5	5	4	2	0.0

* Day 0 denotes fasted body weight the day of dose administration; day 14 denotes final body weight prior to final sacrifice; values in parentheses denote found dead body weights and are not included in the statistical analyses.
- = Not applicable

TABLE 57 (continued)

INDIVIDUAL BODY WEIGHT AND TEST ARTICLE ADMINISTRATION DATA

ACUTE ORAL LD_{50} STUDY IN RATS

TEST ARTICLE: VIOLET MIXTURE

DOSE GROUP: 1,000 mg/kg

Animal Number	Sex	Body Weight (grams) Day of Study* 0	3	4	6	7	10	14	Amount of Test Article Administered (mg)	(ml)
AH3417	M	217	197	-	-	(172)	-	-	218	3.7
AH3418	M	215	198	(195)	-	-	-	-	218	3.7
AH3419	M	230	204	-	(180)	-	-	-	229	3.9
AH3420	M	208	185	(184)	-	-	-	-	206	3.5
AH3421	M	224	201	(198)	-	-	-	-	224	3.8
Mean		219	197						219	3.7
S.D.		8	7						9	0.1
S.E.		4	3						4	0.1
AH2143	F	155	139	-	-	150	158	165	153	2.6
AH2144	F	152	149	-	-	157	165	172	153	2.6
AH2145	F	152	146	-	-	155	165	166	153	2.6
AH2147	F	170	173	-	-	178	184	186	171	2.9
AH2148	F	163	160	-	-	167	173	174	165	2.8
Mean		158	153			161	169	173	159	2.7
S.D.		8	13			11	10	8	8	0.1
S.E.		4	6			5	4	4	4	0.1

* Day 0 denotes fasted body weight the day of dose administration; day 14 denotes final body weight prior to final sacrifice; values in parentheses denote found dead body weights and are not included in the statistical analyses.
- = Not applicable

TABLE 57 (continued)

INDIVIDUAL BODY WEIGHT AND TEST ARTICLE ADMINISTRATION DATA

ACUTE OPAL LD_{50} STUDY IN RATS

TEST ARTICLE: VIOLET MIXTURE

DOSE GROUP: 1,413 mg/kg

Animal Number	Sex	Body Weight (grams) Day of Study* 0	3	3	4	6	7	10	14	Amount of Test Article Administered (mg)	(ml)
AH3412	M	223	(207)	-	-	-	-	-	-	316	3.8
AH3413	M	212	(196)	-	-	-	-	-	-	299	3.6
AH3414	M	222	206	(206)	-	-	-	-	-	316	3.8
AH3415	M	208	(191)	-	-	-	-	-	-	291	3.5
AH3416	M	204	(190)	-	-	-	-	-	-	291	3.5
Mean		214								303	3.6
S.D.		8								13	0.2
S.E.		4								6	0.1
AH1172	F	188	175	-	-	(163)	-	-	-	266	3.2
AH1177	F	173	169	-	-	-	171	180	181	241	2.9
AH1178	F	181	171	-	(164)	-	-	-	-	258	3.1
AH1179	F	196	192	-	-	-	195	190	202	274	3.3
AH1180	F	192	179	-	-	-	174	185	190	274	3.3
Mean		186	177				180	185	191	263	3.2
S.D.		9	9				13	5	11	14	0.2
S.E.		4	4				8	3	6	6	0.1

* Day 0 denotes fasted body weight the day of dose administration; day 14 denotes final body weight prior to final sacrifice; values in parentheses denote found dead body weights and are not included in the statistical analyses.
- = Not applicable

TABLE 57 (continued)

INDIVIDUAL BODY WEIGHT AND TEST ARTICLE ADMINISTRATION DATA

ACUTE ORAL LD_{50} STUDY IN RATS

TEST ARTICLE: *VIOLET MIXTURE*

DOSE GROUP: 1,778 mg/kg

Animal Number	Sex	Body Weight (grams) Day of Study* 0	3	4	Amount of Test Article Administered (mg)	(ml)
AH2155	F	148	(146)	-	261	2.5
AH2156	F	150	148	(144)	272	2.6
AH2157	F	153	(144)	-	272	2.6
AH2158	F	159	(148)	-	282	2.7
AH2159	F	158	(150)	-	282	2.7
Mean		154			274	2.6
S.D.		5			9	0.1
S.E.		2			4	0.0

* Day 0 denotes fasted body weight the day of dose administration; values in parentheses denote found dead body weights and are not included in the statistical analyses.
- = Not applicable

TABLE 57 (continued)

INDIVIDUAL BODY WEIGHT AND TEST ARTICLE ADMINISTRATION DATA

ACUTE ORAL LD_{50} STUDY IN RATS

TEST ARTICLE: VIOLET MIXTURE

DOSE GROUP: 1,995 mg/kg

Animal Number	Sex	Body Weight (grams) Day of Study* 0	2	3	4	Amount of Test Article Administered (mg)	(ml)
AH3422	M	210	(198)	-	-	422	3.6
AH3423	M	212	-	(198)	-	422	3.6
AH3424	M	214	-	(204)	-	422	3.6
AH3425	M	218	-	(207)	-	434	3.7
AH3426	M	227	-	(214)	-	458	3.9
Mean		216				432	3.7
S.D.		7				16	0.1
S.E.		3				7	0.1
AH2150	F	161	-	(152)	-	317	2.7
AH2151	F	152	-	143	(141)	305	2.6
AH2152	F	144	-	(136)	-	282	2.4
AH2153	F	155	-	148	(148)	305	2.6
AH2154	F	168	-	151	(150)	317	2.7
Mean		154		147		305	2.6
S.D.		7		4		14	0.1
S.E.		3		2		6	0.1

* Day 0 denotes fasted body weight the day of dose administration; values in parentheses denote found dead body weights and are not included in the statistical analyses.
- = Not applicable

TABLE 58

INDIVIDUAL ANTEMORTEM OBSERVATIONS

ACUTE ORAL LD_{50} STUDY IN RATS

TEST ARTICLE: VIOLET MIXTURE

DOSE GROUP: 794 mg/kg

Finding	Animal No.: Sex:	AH3435 (M)	AH3436 (M)	AH3437 (M)	AH3438 (M)	AH3440 (M)
		Day(s) Finding Observed				
Purple colored urine		0,1,4,5	0,1,3,4	0,1,3-5	0-5	0-2,4,5
Purple colored loose stools				0		
Purple stained fur - perianal region		1-14	1,3,4	0-14	0-14	1-14
Skin discolored purple		1-3	1-3	1-3	1-3	1-3
Crusty nose		2				
Crusty eye		4-10				
Lethargy		3-5				
Few stools			4			
Sensitive to touch		2				
Death			5			

TABLE 58 (continued)

INDIVIDUAL ANTEMORTEM OBSERVATIONS

ACUTE ORAL LD_{50} STUDY IN RATS

TEST ARTICLE: VIOLET MIXTURE

DOSE GROUP: 1,000 mg/kg

Finding	Animal No.: Sex:	AH3417 (M)	AH3418	AH3419 (M)	AH3420 (M)	AH3421 (M)	AH2143 (F)	AH2144 (F)	AH2145 (F)	AH2147 (F)	AH2148 (F)
		Day(s) Finding Observed									
Purple colored urine		0-6	0-3	0-5	0-4	0-3	0-6	0-6	0-6	0-6	0-6
Skin discolored purple		0-5	0-3	0-5	0-4	0-3	0-4	0-2	0-2	0-2	0-2
Purple stained fur - perianal region		1-6	1-3	1-5	1-4	1-3	1-14	1-14	0-14	0-14	1-14
Loose stools										0	
No stools		0	0	0							
Few stools		2-5	3	2-5			2,3		2		
Ataxia		5,6		5	4						
Lethargy		5,6		5	4						
Crusty eye				4,5	3,4	3	3,4				
Alopecia - posterior legs							10-14				
Death		7	4	6	4	4					

TABLE 58 (continued)

INDIVIDUAL ANTEMORTEM OBSERVATIONS

ACUTE ORAL LD_{50} STUDY IN RATS

TEST ARTICLE: VIOLET MIXTURE

DOSE GROUP: 1,413 mg/kg

		Day(s) Finding Observed									
Finding	Animal No.: Sex:	AH3412 (M)	AH3413 (M)	AH3414 (M)	AH3415 (M)	AH3416 (M)	AH1172 (F)	AH1177 (F)	AH1178 (F)	AH1179 (F)	AH1188 (F)
Purple colored urine		0-3	0-3	0-3	0-2	0-2	0-5	0-6,18	0-3	0-6	0-6
Skin discolored purple		0-3	0-3	0-3	0-2	0-2	0-5	0-4	0-3	0-3	0-4
No stools							0				
Crusty eye										0,1, 4,5	
Purple stained fur - perianal region		1-3	1-3	1-3	1,2	1,2	1-5	1-14	1-3	1-14	1-14
Squinting		2,3	3	3	2		5				
Prostration		3									
Labored respiration		3									
Lethargy			3	3			5				
Ataxia			3	3							
Few stools				1,2			1,4,5				
Lacrimation							5				
Death		3	3	3	3	3	6		4		

TABLE 58 (continued)

INDIVIDUAL ANTEMORTEM OBSERVATIONS

ACUTE ORAL LD_{50} STUDY IN RATS

TEST ARTICLE: VIOLET MIXTURE

DOSE GROUP: 1,778 mg/kg

Finding	Animal No.: Sex:	AH2155 (M)	AH2156 (M)	AH2157 (M)	AH2158 (M)	AH2159 (M)
		Day(s) Finding Observed				
Purple colored urine		0,1	0,1,3	0,1	0-2	? 2
Purple colored loose stools		0				
Purple stained fur - perianal region		0-2	1-3	0,1	1	0-2
Skin discolored purple		1,2	1,2	1,2	1,2	1,2
Prostration			3			
Irregular breathing			3			
Death		3	4	3	3	3

TABLE 58 (continued)

INDIVIDUAL ANTEMORTEM OBSERVATIONS

ACUTE ORAL LD_{50} STUDY IN RATS

TEST ARTICLE: VIOLET MIXTURE

DOSE GROUP: 1,995 mg/kg

					Day(s) Finding Observed					
Finding	Animal No.: Sex:	AH3422 (M)	AH3423 (M)	AH3424 (M)	AH3425 (M)	AH3426 (M)	AH215# (F)	AH2151 (F)	AH2152 (F)	AH2153 (F)	AH2154 (F)
Purple colored urine		0-1	0-2	0-2	0-2	0-2	0-2	0-3	0-2	0-3	0-3
Skin discolored purple		0-1	0-2	0-2	0-2	0-2	0-2	0-3	0-2	0-3	0-2
Purple stained fur - perianal region		1	1,2	1,2	1,2	1,2	1,2	1-3	0-2	1-3	1-3
Few stools			2	2	2		2		2		2,3
No stools						2		3			
Squinting						2					2,3
Crusty eye								3		3	
Prostration										3	3
Body cool to touch										3	3
Death		2	3	3	3	3	3	4	3	4	4

TABLE 59

INDIVIDUAL NECROPSY FINDINGS

ACUTE ORAL LD_{50} STUDY IN RATS

TEST ARTICLE: VIOLET MIXTURE

DOSE GROUP: 794 mg/kg

Animal Number	Sex	Abnormalities Noted at Necropsy (Organ - Abnormality)
AH3435	M	External Surface - Purple stained fur, perianal region Liver - Discoloration, diffuse, dark grey
AH3436*	M	Liver - Discoloration, grey Intestine - Dark contents Fat - Discolored purple Left Rear Appendage - Subcutaneous hemorrhage Stomach - Dark contents Glandular Stomach - Discoloration, multiple, focal, black
AH3437	M	External Surface -- Purple stained fur, perianal region Liver - Discoloration, diffuse, dark grey
AH3438	M	External Surface - Purple stained fur, perianal region Liver - Discoloration, diffuse, dark grey
AH3440	M	External Surface - Purple stained fur, perianal region Liver - Discoloration, diffuse, dark grey

* Animal found dead

TABLE 59 (continued)

INDIVIDUAL NECROPSY FINDINGS

ACUTE ORAL LD_{50} STUDY IN RATS

TEST ARTICLE: VIOLET MIXTURE

DOSE GROUP: 1,000 mg/kg

Animal Number	Sex	Abnormalities Noted at Necropsy (Organ - Abnormality)
AH3417*	M	External Surface - Dried blood around ear tag and muzzle Body Fat - Light purple in color Intestines - Dark contents Liver - Dark with prominent lobular pattern Abdominal Cavity - Small amount of blood Body - Multiple subcutaneous hemorrhages Rear Legs - Large hemorrhages in muscles
AH3418*	M	External Surface - Diffuse, purple, discoloration, fur, skin of pelvic region, tail and appendages Abdominal and Pelvic Fat - Diffuse, purple, discoloration Liver - Diffuse, grey, discoloration; exaggerated lobular pattern Stomach - Dark contents Small Intestine - Dark contents
AH3419*	M	External Surface - Diffuse, purple, discoloration, fur in pelvic region and on tail Liver - Diffuse, grey, discoloration; exaggerated lobular pattern Abdominal and Pelvic Fat - Diffuse, purple, discoloration Stomach - Dark contents Small Intestine - Dark contents
AH3420*	M	External Surface - Diffuse, purple, discoloration, skin and fur of pelvic region, appendages, and tail Abdominal and Pelvic Fat - Diffuse, purple, discoloration Liver - Diffuse, grey, discoloration; exaggerated lobular pattern Stomach - Dark contents Small Intestine - Dark contents Posterior Appendages - Subcutaneous hemorrhage, bilateral

* Animal found dead

TABLE 59 (continued)

INDIVIDUAL NECROPSY FINDINGS

ACUTE ORAL LD_{50} STUDY IN RATS

TEST ARTICLE: VIOLET MIXTURE

DOSE GROUP: 1,000 mg/kg (cont.)

Animal Number	Sex	Abnormalities Noted at Necropsy (Organ - Abnormality)
AH3421*	M	External Surface - Diffuse, purple, discoloration, skin and fur of pelvic region, tail and appendages Abdominal and Pelvic Fat - Diffuse, purple, discoloration Liver - Diffuse, grey, discoloration; exaggerated lobular pattern Stomach - Dark contents Small Intestine - Dark contents
AH2143	F	External Surface - Purple stained fur, perianal region
AH2144	F	External Surface - Purple stained fur, perianal region
AH2145	F	External Surface - Purple stained fur, perianal region
AH2147	F	External Surface - Purple stained fur, perianal region
AH2148	F	External Surface - Purple stained fur, perianal region

* Animal found dead

TABLE 59 (continued)

INDIVIDUAL NECROPSY FINDINGS

ACUTE ORAL LD_{50} STUDY IN RATS

TEST ARTICLE: VIOLET MIXTURE

DOSE GROUP: 1,413 mg/kg

Animal Number	Sex	Abnormalities Noted at Necropsy (Organ - Abnormality)
AH3412*	M	Skin - Purple Stomach - Red foci on glandular stomach Liver - Dark with prominent lobular pattern All Body Fat - Purple Intestines - Purple contents Body - Multiple subcutaneous and muscular hemorrhages throughout
AH3413*	M	Skin - Purple Thoracic Cavity - Blood inside Stomach - Red foci on glandular mucosa Liver - Dark with prominent lobular pattern All Body Fat - Purple Intestines - Purple contents Body - Multiple subcutaneous and muscular hemorrhages throughout Abdominal Cavity - Blood inside
AH3414*	M	Skin - Purple Stomach - Dark contents Intestines - Dark contents Liver - Brown grey discoloration Prostate - Purple Urinary Bladder - Purple fluid contents Fat - Purple Right Posterior Appendage - Subcutaneous hemorrhage
AH3415*	M	Skin - Discolored purple Liver - Brown grey discoloration Stomach - Dark purple contents Intestines - Dark purple contents Urinary Bladder - Purple fluid contents Fat - Discolored purple

* Animal found dead

TABLE 59 (continued)

INDIVIDUAL NECROPSY FINDINGS

ACUTE ORAL LD_{50} STUDY IN RATS

TEST ARTICLE: VIOLET MIXTURE

DOSE GROUP: 1,413 mg/kg (cont.)

Animal Number	Sex	Abnormalities Noted at Necropsy (Organ - Abnormality)
AH3416*	M	Skin - Purple discoloration External Surface - Black crusty material around nose and mouth Lung - Discoloration, red, right middle and left lobes Thymus - Discoloration, dark red Stomach - Dark purple contents Intestine - Dark purple contents Liver - Brown grey discoloration Fat - Purple discoloration Left posterior appendage - Subcutaneous hemorrhage
AH1172*	F	External Surfaces - Discoloration, diffuse, purple, on fur and skin in pelvic region, tail and appendages Abdominal and Pelvic Fat - Discoloration, diffuse, purple Liver - Discoloration, diffuse, grey; exaggerated lobular pattern Stomach - Dark contents Small Intestine - Dark contents
AH1177	F	External Surfaces - Purple stained fur - perineum Liver - Discoloration, diffuse, brown
AH1178*	F	Skin - Purple Liver - Pale; prominent lobular pattern Stomach - Black contents Intestines - Black contents Fat - Purple
AH1179	F	External Surfaces - Purple stained fur - perineum
AH1180	F	External Surfaces - Purple stained fur - perineum Liver - Discoloration, diffuse, brown

* Animal found dead

TABLE 59 (continued)

INDIVIDUAL NECROPSY FINDINGS

ACUTE ORAL LD_{50} STUDY IN RATS

TEST ARTICLE: VIOLET MIXTURE

DOSE GROUP: 1,778 mg/kg (cont.)

Animal Number	Sex	Abnormalities Noted at Necropsy (Organ - Abnormality)
AH2155*	F	External Surfaces - Extremities discolored purple Lung - Mottled red Gastrointestinal Tract - Contains abnormal contents, purple Liver - Prominent lobular pattern; pale Body Fat - Discolored purple Urine - Discolored purple
AH2156*	F	External Surfaces - Extremities discolored purple Lung - Mottled red Gastrointestinal Tract - Contains abnormal contents, purple Liver - Prominent lobular pattern; pale Body Fat - Discolored purple Urine - Discolored purple
AH2157*	F	External Surfaces - Extremities discolored purple Lung - Mottled red Gastrointestinal Tract - Contains abnormal contents, purple Liver - Prominent lobular pattern; pale Body Fat - Discolored purple Urine - Discolored purple
AH2158*	F	External Surfaces - Extremities discolored purple Lung - Mottled red Gastrointestinal Tract - Contains abnormal contents, purple Liver - Prominent lobular pattern; pale Body Fat - Discolored purple Urine - Discolored purple

* Animal found dead

TABLE 59 (continued)

INDIVIDUAL NECROPSY FINDINGS

ACUTE ORAL LD_{50} STUDY IN RATS

TEST ARTICLE: VIOLET MIXTURE

DOSE GROUP: 1,778 mg/kg (cont.)

Animal Number	Sex	Abnormalities Noted at Necropsy (Organ - Abnormality)
AH2159*	F	External Surfaces - Extremities discolored purple Lung - Mottled red Gastrointestinal Tract - Contains abnormal contents, purple Liver - Prominent lobular pattern; pale Body Fat - Discolored purple Urine - Discolored purple

* Animal found dead

TABLE 59 (continued)

INDIVIDUAL NECROPSY FINDINGS

ACUTE ORAL LD_{50} STUDY IN RATS

TEST ARTICLE: VIOLET MIXTURE

DOSE GROUP: 1,995 mg/kg (cont.)

Animal Number	Sex	Abnormalities Noted at Necropsy (Organ - Abnormality)
AH3422*	M	Skin - Purple Stomach - Black contents Intestines - Black contents Liver - Brown grey discoloration Urinary Bladder - Purple fluid contents Fat - Purple
AH3423*	M	Skin - Purple Stomach - Purple to black contents Intestines - Purple to black contents Liver - Grey brown; exaggerated lobular pattern Urinary Bladder - Purple fluid contents Fat - Purple
AH3424*	M	Skin - Purple Stomach - Purple to black contents Intestines - Purple to black contents Liver - Grey brown; exaggerated lobular pattern Testes - Purple Urinary Bladder - Purple fluid contents Fat - Purple
AH3425*	M	Skin - Purple Stomach - Black contents Intestines - Black contents Liver - Grey brown; exaggerated lobular pattern Urinary Bladder - Purple fluid contents Fat - Purple
AH3426*	M	Skin - Purple Stomach - Purple to black contents Intestines - Purple to black contents Liver - Grey brown; exaggerated lobular pattern Testes - Purple Urinary Bladder - Purple fluid contents Fat - Purple

* Animal found dead

TABLE 59 (continued)

INDIVIDUAL NECROPSY FINDINGS

ACUTE ORAL LD_{50} STUDY IN RATS

TEST ARTICLE: VIOLET MIXTURE

DOSE GROUP: 1,995 mg/kg (cont.)

Animal Number	Sex	Abnormalities Noted at Necropsy (Organ - Abnormality)
AH2150*	F	Skin - Purple Stomach - Purple to black contents Intestines - Purple to black contents Liver - Exaggerated lobular pattern Urinary Bladder - Purple fluid contents Fat - Purple Abdominal Cavity - Red fluid inside
AH2151*	F	External Surfaces - Discoloration, diffuse, purple, on fur and skin in pelvic region, tail and appendages Abdominal and Pelvic Fat - Discoloration, diffuse, purple Liver - Discoloration, diffuse, grey; exaggerated lobular pattern Stomach - Dark contents Small Intestine - Dark contents
AH2152*	F	Skin - Purple Intestine - Purple Liver - Exaggerated lobular pattern Stomach - Purple to black contents Intestines - Purple to black contents Fat - Purple
AH2153*	F	External Surfaces - Discoloration, diffuse, purple, on fur and skin in pelvic region, tail and appendages Abdominal and Pelvic Fat - Discoloration, diffuse, purple Liver - Discoloration, diffuse, grey; exaggerated lobular pattern Stomach - Dark contents Small Intestine - Dark contents

* Animal found dead

TABLE 59 (continued)

INDIVIDUAL NECROPSY FINDINGS

ACUTE ORAL LD_{50} STUDY IN RATS

TEST ARTICLE: VIOLET MIXTURE

DOSE GROUP: 1,995 mg/kg (cont.)

Animal Number	Sex	Abnormalities Noted at Necropsy (Organ - Abnormality)
AH2154*	F	External Surfaces - Discolorations, diffuse, purple, on fur and skin in pelvic region, tail and appendages Abdominal and Pelvic Fat - Discoloration, diffuse, purple Liver - Exaggerated lobular pattern Stomach - Dark contents Small Intestine - Dark contents

* Animal found dead

TABLE 60

LITCHFIELD-WILCOXON LD_{50} FOR MALES

ACUTE ORAL LD_{50} STUDY IN RATS

TEST ARTICLE: VIOLET MIXTURE

Dose (mg/kg)	Observed Deaths Proportion	Percent	Expected Deaths Percent	Difference
794.0	1/5	20.0		
1,000.0	5/5	100.0		
1,413.0	5/5	100.0		
1,995.0	5/5	100.0		

Total number of animals: 20

At least two dose levels with percent observed death between 0 and 100 are required to calculate a least-squares regression. The LD_{50} cannot be computed.

TABLE 61

LITCHFIELD-WILCOXON LD_{50} FOR FEMALES

ACUTE ORAL LD_{50} STUDY IN RATS

TEST ARTICLE: VIOLET MIXTURE

Dose (mg/kg)	Observed Deaths Proportion	Observed Deaths Percent	Expected Deaths Percent	Difference
1,000.0	0/5	0.0		
1,413.0	2/5	40.0		
1,778.0	5/5	100.0		
1,995.0	5/5	100.0		

Total number of animals: 20

At least two dose levels with percent observed death between 0 and 100 are required to calculate a least-squares regression. The LD_{50} cannot be computed.

TABLE 62

LITCHFIELD-WILCOXON LD_{50} FOR COMBINED SEXES

ACUTE ORAL LD_{50} STUDY IN RATS

TEST ARTICLE: VIOLET MIXTURE

Dose (mg/kg)	Observed Deaths Proportion	Observed Deaths Percent	Expected Deaths Percent	Difference
794.0	1/5	20.0	20.1	-0.1
1,000.0	5/10	50.0	44.0	6.0
1,413.0	7/10	70.0	81.0	-11.0
1,778.0	5/5	100.0(95.9)	94.1	1.8
1,995.0	10/10	100.0(97.4)	97.2	0.2

Total number of animals: 40

NOTE - The values in parentheses are those used by the Litchfield-Wilcoxon method to compute Chi-Square contributions.

Calculated Chi-Square: 0.802

Critical Chi-Square (P = .05) for 3 degrees of freedom: 7.812

The data are not significantly heterogeneous.

Calculated LD-50: 1052.0 mg/kg

95% Confidence Limits: 873.6 - 1266.8 mg/kg

The confidence limits are within 20.4% of the LD-50.

Slope: 6.87 (probits/log dose)

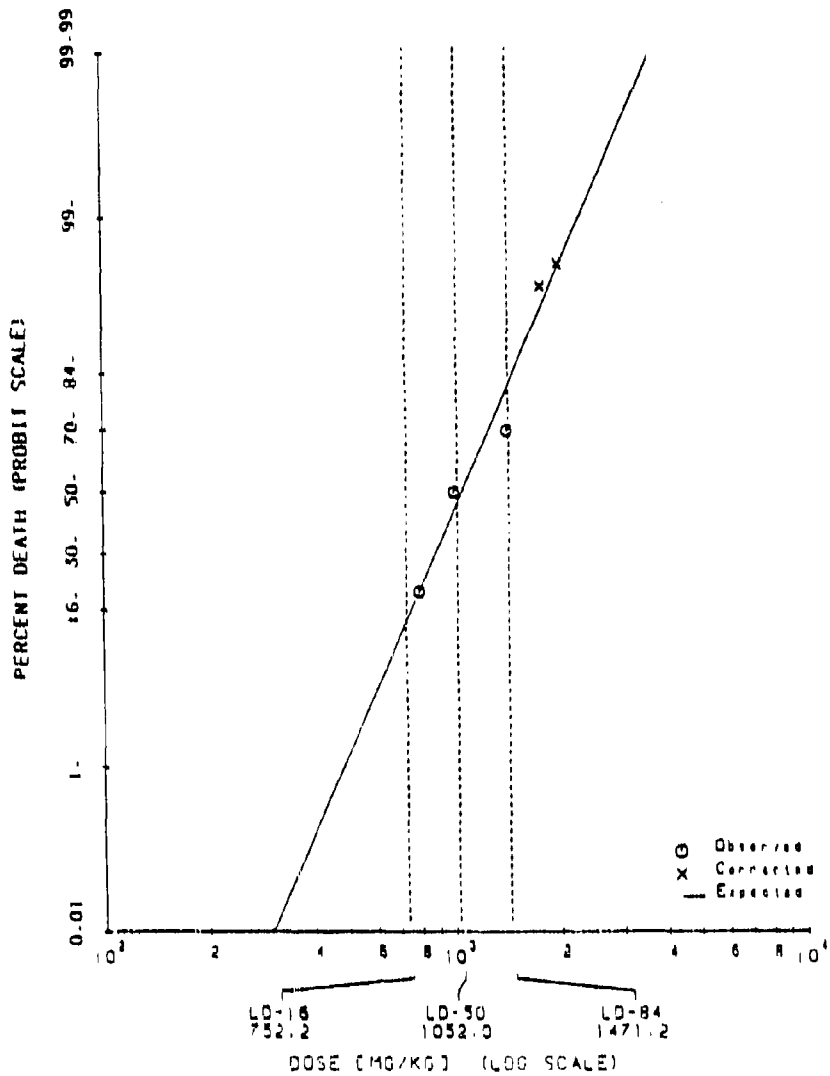
There are 25 animals included in groups with expected deaths between 16% (LD-16 = 752.2 mg/kg) and 84% (LD-84 = 1471.2 mg/kg).

Given the slope calculated from the present data, a total of 26 animals would be needed in groups with expected deaths between 16% and 84% in order to get the confidence limits within 20% of the LD-50. However, adding more test groups may change the value of the slope.

FIGURE E-2: DOSE-RESPONSE CURVE FOR COMBINED SEXES

ACUTE ORAL LD_{50} STUDY IN RATS

TEST ARTICLE: VIOLET MIXTURE



American Biogenics Corporation

APPENDIX A

QUALITY ASSURANCE INSPECTIONS AND AUDITS

QUALITY ASSURANCE INSPECTIONS AND AUDITS

Study	Type of Inspection/ Audit	Parameter	Date(s) Conducted Start	Finish	Date Reported to Management
480-2270	Report	Status	9/09/85	9/09/85	9/09/85
	Data	All	2/11/86	2/14/86	2/14/86
	Report	Draft	2/11/86	2/14/86	2/14/86
	Report	Final	8/29/86	8/29/86	8/29/86
480-2271	Report	Status	9/09/85	9/09/85	9/09/85
	Data	All	2/11/86	2/14/86	2/14/86
	Report	Draft	2/11/86	2/14/86	2/14/86
	Report	Final	8/29/86	8/29/86	8/29/86
480-2272	Report	Status	9/30/85	9/30/85	9/30/85
	Report	Status	1/31/86	1/31/86	1/31/86
	Data	All	2/11/86	2/14/86	2/14/86
	Report	Draft	2/11/86	2/14/86	2/14/86
	Report	Final	8/29/86	8/29/86	8/29/86
480-2273	Data	All	2/11/86	2/14/86	2/14/86
	Report	Draft	2/11/86	2/14/86	2/14/86
	Report	Final	8/29/86	8/29/86	8/29/86
480-2274	Data	All	2/11/86	2/14/86	2/14/86
	Report	Draft	2/11/86	2/14/86	2/14/86
	Report	Final	8/29/86	8/29/86	8/29/86
480-2275	Data	All	2/11/86	2/14/86	2/14/86
	Report	Draft	2/11/86	2/14/86	2/14/86
	Report	Final	8/29/86	8/29/86	8/29/86
480-2276	Report	Status	10/02/85	10/02/85	10/02/85
	Report	Status	1/20/86	1/20/86	1/20/86
	Data	All	2/11/86	2/14/86	2/14/86
	Report	Draft	2/11/86	2/14/86	2/14/86
	Report	Final	8/29/86	8/29/86	8/29/86
480-2277	Report	Status	11/06/85	11/06/85	11/06/85
	Data	All	2/11/86	2/14/86	2/14/86
	Report	Draft	2/11/86	2/14/86	2/14/86
	Report	Final	8/29/86	8/29/86	8/29/86
480-2278	Data	All	2/11/86	2/14/86	2/14/86
	Report	Draft	2/11/86	2/14/86	2/14/86
	Report	Final	8/29/86	8/29/86	8/29/86
480-2279	Data	All	2/11/86	2/14/86	2/14/86
	Report	Draft	2/11/86	2/14/86	2/14/86
	Report	Final	8/29/86	8/29/86	8/29/86

QUALITY ASSURANCE INSPECTIONS AND AUDITS

Study	Type of Inspection/ Audit	Parameter	Date(s) Conducted Start	Finish	Date Reported to Management
480-2280	Report	Status	9/30/85	9/30/85	9/30/85
	Data	All	2/11/86	2/14/86	2/14/86
	Report	Draft	2/11/86	2/14/86	2/14/86
	Report	Final	8/29/86	8/29/86	8/29/86
480-2281	Report	Status	11/06/85	11/06/85	11/06/85
	Data	All	2/11/86	2/14/86	2/14/86
	Report	Draft	2/11/86	2/14/86	2/14/86
	Report	Final	8/29/86	8/29/86	8/29/86
480-2282	Report	Status	10/22/85	10/22/85	10/22/85
	Data	All	2/11/86	2/14/86	2/14/86
	Report	Draft	2/11/86	2/14/86	2/14/86
	Report	Final	8/29/86	8/29/86	8/29/86
480-2283	Report	Status	10/22/85	10/22/85	10/22/85
	Data	All	2/11/86	2/14/86	2/14/86
	Report	Draft	2/11/86	2/14/86	2/14/86
	Report	Final	8/29/86	8/29/86	8/29/86
480-2284	Report	Status	11/13/85	11/13/85	11/13/85
	Data	All	2/11/86	2/14/86	2/14/86
	Report	Draft	2/11/86	2/14/86	2/14/86
	Report	Final	8/29/86	8/29/86	8/29/86
480-2285	Report	Status	1/30/86	1/30/86	1/30/86
	Data	All	2/11/86	2/14/86	2/14/86
	Report	Draft	2/11/86	2/14/86	2/14/86
	Report	Final	8/29/86	8/29/86	8/29/86
480-2286	Report	Status	9/24/85	9/24/85	9/24/85
	Data	All	2/11/86	2/14/86	2/14/86
	Report	Draft	2/11/86	2/14/86	2/14/86
	Report	Final	8/29/86	8/29/86	8/29/86
480-2287	Report	Status	9/27/85	9/27/85	9/27/85
	Data	All	2/11/86	2/14/86	2/14/86
	Report	Draft	2/11/86	2/14/86	2/14/86
	Report	Final	8/29/86	8/29/86	8/29/86
480-2288	Report	Status	10/04/85	10/04/85	10/04/85
	Report	Status	11/21/85	11/21/85	11/21/85
	Data	All	2/11/86	2/14/86	2/14/86
	Report	Draft	2/11/86	2/14/86	2/14/86
	Report	Final	8/29/86	8/29/86	8/29/86

QUALITY ASSURANCE INSPECTIONS AND AUDITS

Study	Type of Inspection/ Audit	Parameter	Date(s) Conducted Start	Finish	Date Reported to Management
480-2289	Report	Status	11/06/85	11/06/85	11/06/85
	Data	All	2/11/86	2/14/86	2/14/86
	Report	Draft	2/11/86	2/14/86	2/14/86
	Report	Final	8/29/86	8/29/86	8/29/86
480-2290	Report	Status	10/22/85	10/22/85	10/22/85
	Data	All	2/11/86	2/14/86	2/14/86
	Report	Draft	2/11/86	2/14/86	2/14/86
	Report	Final	8/29/86	8/29/86	8/29/86
480-2291	Report	Status	11/04/85	11/04/85	11/04/85
	Data	All	2/11/86	2/14/86	2/14/86
	Report	Draft	2/11/86	2/14/86	2/14/86
	Report	Final	8/29/86	8/29/86	8/29/86
480-2292	Report	Status	11/12/85	11/12/85	11/12/85
	Data	All	2/11/86	2/14/86	2/14/86
	Report	Draft	2/11/86	2/14/86	2/14/86
	Report	Final	8/29/86	8/29/86	8/29/86
480-2293	Report	Status	1/30/86	1/30/86	1/30/86
	Data	All	2/11/86	2/14/86	2/14/86
	Report	Draft	2/11/86	2/14/86	2/14/86
	Report	Final	8/29/86	8/29/86	8/29/86

APPENDIX B

GRADING SYSTEM FOR EVALUATION OF DERMAL REACTIONS

APPENDIX B

GRADING SYSTEM FOR EVALUATION OF DERMAL REACTIONS+

(1) Erythema and Eschar Formation

No erythema	Ø
Very slight erythema (barely perceptible)	1
Well defined erythema	2
Moderate to severe erythema	3
Severe erythema (beet redness) to slight eschar formation (injuries in depth)	4
Total possible erythema score	4

(2) Edema Formation

No edema	Ø
Very slight edema (barely perceptible)	1
Slight edema (edges of area well defined by definite raising)	2
Moderate edema (raised approximately 1 mm)	3
Severe edema (raised more than 1 mm and extending beyond area of exposure)	4
Total possible edema score	4

Other dermal reactions observed were also recorded.

+ Draize, J. H., "Appraisal of the Safety of Chemicals in Foods, Drugs, and Cosmetics", The Association of Food and Drug Officials of the United States, Fourth Printing, 1979, p. 48.

APPENDIX C

GRADING SYSTEM FOR EVALUATION OF EYE IRRITATION

APPENDIX C

GRADING SYSTEM FOR EVALUATION OF EYE IRRITATION+

I. Cornea

A. Opacity-Degree of Density (area most dense taken for reading)

No opacity	0
Scattered or diffuse area, details of iris clearly visible	(1)*
Easily discernible translucent areas, details of iris slightly obscured	2
Opalescent areas, no details of iris visible, size of pupil barely discernible	3
Opaque, iris invisible	4

B. Area of Cornea Involved

One-quarter (or less) but not zero	1
Greater than one-quarter, but less than one-half	2
Greater than one-half, but less than three-quarters	3
Greater than three-quarters, up to whole area	4

A x B x 5 Total Maximum = 80

II. Iris

A. Values

Normal	0
Folds above normal, congestion, swelling, circumcorneal injection (any or all of these or combination of any thereof) iris still reacting to light (sluggish reaction is positive)	(1)*
No reaction to light, hemorrhage, gross destruction (any or all of these)	2

A x 5 Total Maximum = 10

III. Conjunctivae

A. Redness (refers to palpebral and bulbar conjunctivae excluding cornea and iris)

Vessels normal	0
Vessels definitely injected above normal	1
More diffuse, deeper crimson red, individual vessels not easily discernible	(2)*
Diffuse beefy red	3

APPENDIX C

GRADING SYSTEM FOR EVALUATION OF EYE IRRITATION+

B. Chemosis

No swelling	0
Any swelling above normal (includes nictitating membrane)	1
Obvious swelling with partial eversion of the lids	(2)*
Swelling with lids about half closed	3
Swelling with lids about half closed to completely closed.	4

C. Discharge

No discharge	0
Any amount different from normal (does not include small amount observed in inner canthus of normal animals)	1
Discharge with moistening of the lids and hairs just adjacent to the lids	2
Discharge with moistening of the lids and considerable area around the eye	3

(A + B + C) x 2 Total Maximum = 20

Total Possible Score = I + II + III = 110

IV. Fluorescein Stain Retention: Not in the Draize Table and not included in the Primary Eye Irritation Scores. Any stain retention was considered to be epithelial swelling/erosion and not true stromal opacity.

Area of Cornea Involved

None	0
One-quarter (or less) but not zero	1
Greater than one-quarter, but less than one-half	2
Greater than one-half, but less than three-quarters	3
Greater than three-quarters, up to whole area	4

* Bracketed figures indicate lowest grades considered positive.

+ Draize, J. H., "Appraisal of the Safety of Chemicals in Foods, Drugs, and Cosmetics", The Association of Food and Drug Officials of the United States, Fourth Printing, 1979, p. 51.

APPENDIX D

PATHOLOGY REPORTS

Key:

AMERICAN BIOGENICS STUDY NO.	TEST ARTICLE
480-2273	Disperse Red 11 - Lot 1
480-2277	Disperse Red 11 - Lot 2
480-2281	Disperse Blue 3
480-2285	Violet Mixture - 35 parts Disperse Red 11 (Lot 1) to 5 parts Disperse Blue 3
480-2289	Solvent Red 1
480-2293	Red Mixture - 33.4 parts Solvent Red 1 to 6.6 parts Disperse Red 11 (Lot 1)

EPL

EXPERIMENTAL PATHOLOGY LABORATORIES, INC.
1800 EAST PERSHING ROAD, DECATUR, ILLINOIS 62526 (217) 875-3930

October 30, 1985

Dr. Dale Mayhew
American Biogenics Corporation
1800 E. Pershing Road
Decatur, IL 62526

Reference: Study Number 480-2273

Dear Dr. Mayhew:

In regards to the referenced study, treated and untreated skin were microscopically examined from 4 rabbits (BB9414, BB9413, BB9432, and BB9433). All tissues were found to be not remarkable. No histopathological abnormalities were seen that could be associated with any macroscopic observation.

If I can be of further assistance to you, please do not hesitate to contact me.

Cordially,

Stephen V. Becker, D.V.M.

SVB/jcs

EPL

EXPERIMENTAL PATHOLOGY LABORATORIES, INC.

QUALITY ASSURANCE REPORT CERTIFICATION

Client Name: American Biogenics Corporation

Client Study Number: 480-2273

Study Director: Dr. W.O. Iverson

Pathologist: Dr. S.V. Becker

Study Title: Acute Dermal Toxicity Study in Rabbits

Test Article: Disperse Red II, lot 1

Species: Albino rabbit

All parts of the pathology phase of this study, including the final report, were reviewed by Experimental Pathology Laboratories Quality Assurance Unit on October 29, 1985. All findings were reported to the Study Director and Management.

Patricia S. Malone

Patricia S. Malone, B.S.

10/31/85

EPL

EXPERIMENTAL PATHOLOGY LABORATORIES, INC.
1800 EAST PERSHING ROAD, DECATUR, ILLINOIS 62526 (217) 875-3930

October 30, 1985

Dr. Dale Mayhew
American Biogenics Corporation
1800 E. Pershing Road
Decatur, IL 62526

Reference: Study Number 480-2277

Dear Dr. Mayhew:

In regards to the referenced study, treated and untreated skin were microscopically examined from 5 rabbits (BB9424, BB9454, BB9447, BB9428, and BB9425). All tissues were found to be not remarkable. No histopathological abnormalities were seen that could be associated with any macroscopic observation.

If I can be of further assistance to you, please do not hesitate to contact me.

Cordially,

Stephen V. Becker, D.V.M.

SVB/jcs

EPL

EXPERIMENTAL PATHOLOGY LABORATORIES, INC.

QUALITY ASSURANCE REPORT CERTIFICATION

Client Name: American Biogenics Corporation

Client Study Number: 480-2277

Study Director: Dr. W.O. Iverson

Pathologist: Dr. S.V. Becker

Study Title: Acute Dermal Toxicity Study in Rabbits

Test Article: Disperse Red II, lot 2

Species: Albino rabbit

All parts of the pathology phase of this study, including the final report, were reviewed by Experimental Pathology Laboratories Quality Assurance Unit on October 29, 1985. All findings were reported to the Study Director and Management.

Patricia S. Malone

Patricia S. Malone, B.S.

10/31/85

EPL

EXPERIMENTAL PATHOLOGY LABORATORIES, INC.
1800 EAST PERSHING ROAD, DECATUR, ILLINOIS 62526 (217) 875-3930

October 30, 1985

Dr. Dale Mayhew
American Biogenics Corporation
1800 E. Pershing Road
Decatur, IL 62526

Reference: Study Number 480-2281

Dear Dr. Mayhew:

In regards to the referenced study, treated and untreated skin were microscopically examined from 4 rabbits (BB9450, BB9448, BB9412, and BB9410). All tissues were found to be not remarkable. No histopathological abnormalities were seen that could be associated with any macroscopic observation.

If I can be of further assistance to you, please do not hesitate to contact me.

Cordially,

Stephen V. Becker, D.V.M.

SVB/jcs

EPL

EXPERIMENTAL PATHOLOGY LABORATORIES, INC.

QUALITY ASSURANCE REPORT CERTIFICATION

Client Name: American Biogenics Corporation

Client Study Number: 480-2281

Study Director: Dr. W.O. Iverson

Pathologist: Dr. S.V. Becker

Study Title: Acute Dermal Toxicity Study in Rabbits

Test Article: Disperse Blue #3

Species: Albino rabbit

All parts of the pathology phase of this study, including the final report, were reviewed by Experimental Pathology Laboratories Quality Assurance Unit on October 29, 1985. All findings were reported to the Study Director and Management.

Patricia S. Malone, B.S.

10/31/85

EPL

EXPERIMENTAL PATHOLOGY LABORATORIES, INC.
1800 EAST PERSHING ROAD, DECATUR, ILLINOIS 62526 (217) 875-3930

November 20, 1985

Dr. Dale Mayhew
American Biogenics Corporation
1800 E. Pershing Road
Decatur, IL 62526

Reference: Study Number 480-2285

Dear Dr. Mayhew:

In regards to the referenced study, treated and untreated skin were microscopically examined from 4 rabbits (BB9426, BB9597, BB9626, and BB9645). The results of the evaluation were as follows:

BB9426	Control-Not Remarkable Test-Not Remarkable
BB9597	Control-Focal Chronic Dermatitis, Minimal Test-Not Remarkable
BB9626	Control-Hemorrhage, Dermis, Mild, Acute Test-Hemorrhage, Dermis, Minimal, Acute
BB9645	Control-Not Remarkable Test-Not Remarkable

No histopathological abnormalities were seen that could be associated with any macroscopic observation or with the test article.

If I can be of further assistance to you, please do not hesitate to contact me.

Cordially,

Stephen V. Becker, D.V.M.

SVB/jcs

QUALITY ASSURANCE
REPORT CERTIFICATION

Client Name: American Biogenics Corporation

Client Study Number: 480-2285

Study Director: Dr. W.O. Iverson

Pathologist: Dr. S.V. Becker

Study Title: Acute Dermal Toxicity Study in Rabbits

Test Article: Violet Mixture

Species: Albino rabbit

All parts of the pathology phase of this study, including the final report, were reviewed by Experimental Pathology Laboratories Quality Assurance Unit on October 29, November 3, 13, and 19, 1985. All findings were reported to the Study Director and Management.

Patricia S. Malone, B.S.

11/21/85

EPL

EXPERIMENTAL PATHOLOGY LABORATORIES, INC.
1800 EAST PERSHING ROAD, DECATUR, ILLINOIS 62526 (217) 875-3930

October 30, 1985

Dr. Dale Mayhew
American Biogenics Corporation
1800 E. Pershing Road
Decatur, IL 62526

Reference: Study Number 480-2289

Dear Dr. Mayhew:

In regards to the referenced study, treated and untreated skin were microscopically examined from 4 rabbits (BB9477, BB9484, BB9535, and BB9536). All tissues were found to be not remarkable. No histopathological abnormalities were seen that could be associated with any macroscopic observation.

If I can be of further assistance to you, please do not hesitate to contact me.

Cordially,

Stephen V. Becker, D.V.M.

SVB/jcs

EPL

EXPERIMENTAL PATHOLOGY LABORATORIES, INC.

QUALITY ASSURANCE
REPORT CERTIFICATION

Client Name: American Biogenics Corporation

Client Study Number: 480-2289

Study Director: Dr. W.O. Iverson

Pathologist: Dr. S.V. Becker

Study Title: Acute Dermal Toxicity Study in Rabbits

Test Article: Solvent Red 1

Species: Albino rabbit

All parts of the pathology phase of this study, including the final report, were reviewed by Experimental Pathology Laboratories Quality Assurance Unit on October 29, 1985. All findings were reported to the Study Director and Management.

Patricia S. Malone

Patricia S. Malone, B.S.

10/31/85

EPL
EXPERIMENTAL PATHOLOGY LABORATORIES, INC.
1800 EAST PERSHING ROAD, DECATUR, ILLINOIS 62526 (217) 875-3930

December 16, 1985

Dr. Dale Mayhew
American Biogenics Corporation
1800 E. Pershing Road
Decatur, IL 62526

Reference: Study Number 480-2293

Dear Dr. Mayhew:

In regards to the referenced study, treated and untreated skin were microscopically examined from 4 rabbits (BB9789, BB9800, BB9817, and BB9824). All tissues were found to be not remarkable. No histopathological abnormalities were seen that could be associated with any macroscopic observation or with the test article.

If I can be of further assistance to you, please do not hesitate to contact me.

Cordially,

Stephen V. Becker, D.V.M.

SVB/jcs

QUALITY ASSURANCE
REPORT CERTIFICATION

Client Name: American Biogenics Corporation

Client Study Number: 480-2293

Study Director: Dr. W.O. Iverson

Pathologist: Dr. S.V. Becker

Study Title: Acute Dermal Toxicity Study in Rabbits

Test Article: Red Mixture

Species: Albino rabbit

All parts of the pathology phase of this study, including the final report, were reviewed by Experimental Pathology Laboratories Quality Assurance Unit on December 1, 9 and 12, 1985. All findings were reported to the Study Director and Management.

Patricia S. Malone

Patricia S. Malone, B.S.

12/16/85

APPENDIX E

LIST OF PERSONNEL

PERSONNEL

American Biogenics personnel listed below participated in the conduct and/or report preparation of these studies:

Gary L. Doyle, B.S.	Acute Department, Technician
Jonathan C. Kreuger, B.A.	Acute Department, Technician
Kathy Mellon, M.S.	Acute Department, Technician
Sandra H. Smith	Acute Department, Project Supervisor
Dale A. Mayhew, Ph.D.	Director, Toxicology and Principle Investigator
Stephen V. Becker, D.V.M.	Experimental Pathology Laboratories, Pathologist
William O. Iverson, D.V.M.	Experimental Pathology Laboratories, Director of Pathology Services
Antoinette Skelley	Manager, Quality Assurance, Regulatory Affairs, and Archives

APPENDIX F

DISTRIBUTION LIST

DISTRIBUTION LIST
FINAL REPORTS FOR SMOKE/OBSCURANTS

Number of copies	Address
6	Project manager for Smoke/Obscurants Bldg. 324 ATTN: AMCPM-SMK-E (Dr. Lock) Aberdeen Proving Ground, MD 21005-5001
1	Commander/Director Chemical Research, Development and Engineering Center ATTN: SMCCR-MUS-P (Mr. Young) Aberdeen Proving Ground, MD 21010-5423
1	Commander/Director Chemical Research, Development and Engineering Center ATTN: SMCCR-RST-E (Mr. Weimer) Aberdeen Proving Ground, MD 21010-5423
1	Officer-in-Charge Naval Medical Research Institute Toxicology Detachment Building 433 Wright-Patterson AFB, OH 45433
1	HQDA (DASG-PSP-O) 5111 Leesburg Pike Falls Church, VA 22041-3258
1	Commander US Air Force Aerospace Medical Research Laboratory ATTN: Toxic Hazards Division Bldg. 79, Area B Wright-Patterson AFB, OH 45433
1	Commander US Army Health Services Command ATTN: HSCL-P Fort Sam Houston, TX 78234-6000
1	Commander US Army Armament Munitions & Chemical Command ATTN: AMSMC-SG Rock Island, IL 61299

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1 Commander
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